



Final Technical Report

2011

Central Valley Bacteria Source Identification Screening Study (Source ID Study)

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1.0 EXECUTIVE SUMMARY

The objective of this study was to perform a preliminary investigation of the occurrence of bacteria and evaluate potential sources throughout the Central Valley. Specific objectives were to:

- Evaluate seasonal bacteria concentrations and trends in selected water bodies within the Central Valley of California,
- Determine whether *E. coli* O157:H7 and/or *Salmonella* are present at any time at the sites being evaluated,
- Evaluate potential sources of fecal contamination and group potential sources to human, cattle, or other animals,
- Document the presence of source identifier DNA in viable vs. non-viable *Bacteroidales* cells in relation to the presence of *E. coli*, *E. coli* O157:H7, and *Salmonella*,
- Compare reported concentrations to appropriate water quality objectives and guidelines including the Central Valley Regional Board Basin Plan (Basin Plan, 2007) and the USEPA Bacterial Water Quality Standards for Recreational Waters guidelines (USEPA Standards, 2003),
- Make recommendations for future bacteria source identification studies.

The Monitoring Plan was designed to include sites throughout the Central Valley, which were potentially influenced by a variety of bacteria loading sources. Twelve study sites were selected in watersheds within the Sacramento River and San Joaquin River basins:

Sacramento River basin

- American River at Discovery Park (Sacramento County)
- Colusa Basin Drain above Knights Landing (Yolo County)
- Dry Creek at Cirby Creek confluence (Placer County)
- Elder Creek at Gerber (Tehama County)
- Upper Sacramento River at Red Bluff (Tehama County)

San Joaquin River basin

- Bear Creek at Lower Sacramento Rd (San Joaquin County)
- Dry Creek at La Loma Road (Stanislaus County)
- Harding Drain discharge to San Joaquin River (Stanislaus County)
- Lone Tree Creek at Brennan Road (San Joaquin County)
- Mokelumne River at New Hope Road (Sacramento County)
- Sutter Creek at Hwy 49 (Amador County)
- Woods Creek at Motherlode Fairgrounds (Tuolumne County)

Sampling events were scheduled to allow for seasonal comparison of results. Between May and December 2009, staff from the Central Valley Regional Water Quality Control Board (Central Valley Water Board) conducted 4 sampling events: May 26, July 20, October 19, and December 14, 2009, representing spring runoff, summer irrigation, fall

dry (note that it rained during this sampling event), and winter runoff seasons, respectively.

Field measurements included water temperature, specific conductivity, dissolved oxygen, pH, photo monitoring, and turbidity. Water samples were collected for analysis of *E. coli*, *E. coli* O157:H7, *Salmonella* spp, *Bacteroidales*, and *Bacteroidale* viability. *E. coli* was analyzed in the Central Valley Water Board laboratory. *E. coli* O157:H7 and *Salmonella* were analyzed at the Western Institute for Food Safety and Security and the Atwill Laboratory, located in the Department of Population Health and Reproduction, School of Veterinary Medicine at the University of California, Davis. *Bacteroidales* and viability were analyzed at the Wuertz Laboratory, Department of Civil and Environmental Engineering at University of California, Davis.

Summary Results

Field parameters varied with seasons and sample sites. In general, water temperature tended to be higher in spring runoff and irrigation seasons and lower in fall dry and winter runoff seasons. Dissolved oxygen tends to be lower during peaks in temperature. This trend was observed during this study, with anomalies at Sacramento River below Red Bluff and Bear Creek at Lower Sacramento Road during the irrigation season. The lowest pH was observed in fall dry season in all sampling sites. No apparent trends of specific conductivity and turbidity were observed during the four sampling seasons.

E. coli were detected from all sampling sites during all sampling seasons. Though concentrations of *E. coli* vary with sampling sites and seasons, no significant associations with season were found based on statistical analysis. However, observation of summary data indicates concentrations were highest during the irrigation and winter runoff seasons.

E. coli O157:H7 was detected in two of the 48 water samples collected during the entire project. The first positive sample occurred in May 2009 from Lone Tree Creek at Brennan Road, San Joaquin River basin, in San Joaquin County. This water sample also had the highest level of indicator *E. coli* during the project (>2419.6 MPN/100 ml), tested positive for *Salmonella*, and also tested positive for cow and dog fecal sources. The other positive sample occurred in July 2009 from the Sacramento River below Red Bluff in Tehama County. Interestingly, and quite opposite the trends of site 13, this water sample had very low levels of indicator *E. coli* (16 MPN/100 ml), tested negative for *Salmonella*, and did not have a species-specific fecal source detected by the *Bacteroidales* method.

Salmonella was detected in about a third (35%) of the samples from the Sacramento basin sites and about half (48%) of the San Joaquin sampling sites, with concentrations ranging from 0.09 to 2.7 MPN/100 ml. In Sacramento River watersheds, all sites had at least one season that tested positive for *Salmonella*. Seasonal occurrence (MPN>0) at the 5 sites was 1/5, 0/5, 3/5, and 2/5 for spring runoff, irrigation, dry and winter runoff seasons, respectively. The mean concentration was 0.24, 0, 0.81, and 0.09 MPN/100ml

for spring runoff, irrigation, dry and winter runoff seasons, respectively. In the San Joaquin River basin, the majority of sites were positive for *Salmonella* during one or more seasons. Seasonal occurrence at the 7 sites was 2/7, 3/7, 4/7, and 4/7 for spring runoff, irrigation, dry and winter runoff seasons, respectively. The mean concentration was 0.12, 0.29, 0.10, and 0.13 MPN/100ml for the same 4 seasons, respectively.

Almost all water samples (98%) had the universal fecal source *Bacteroidales* marker. About 6% of the water samples from the Sacramento River basin monitoring sites had detectable human fecal sources; similarly, about 6% of water samples had cow fecal sources. About 11% and 37% of water samples had human and cow fecal sources, respectively, for the San Joaquin watershed monitoring sites. The procedure that distinguishes viable from non-viable *Bacteroidales* generated unreliable data so the decision was made not to interpret that portion of the data. The Wuertz laboratory is in the process of resolving the issue for future analyses.

The relatively frequent occurrence of human fecal sources (3/4 positive) at Woods Creek at Mother Lode Fairgrounds and cow fecal sources (4/4 positive) at Lone Tree Creek at Brennan Road are supportive that human and bovine activity upstream of these monitoring sites may be contributing a significant portion of the microbial species (*E. coli* O157:H7, *Salmonella*) and indicator *E. coli* isolated from these locations. Moreover, the persistent and high concentrations of *E. coli* at Lone Tree Creek on Brennan Road warrant a more in-depth investigation as to the cause of the water quality impairment. This site was one of the few sites that tested positive for *E. coli* O157:H7 and had persistent levels of *Salmonella*.

Indicators of Beneficial Use protection included the Central Valley Water Board Basin Plan, Calfed Guidelines, USEPA Criteria, State Water Resources Control Board Objectives, and recommendations from the Food and Agriculture Organization of the United Nations and Water Quality for Agriculture. Beneficial uses evaluated were drinking water (pH, SC, *E. coli*), irrigation water supply (SC), aquatic life (water temperature, DO, pH, and turbidity), and Recreation (*E. coli*). All results for pH and SC were within the drinking water recommendations, however *E. coli* was present in all samples at all sites, supporting the recommendation for treatment prior to drinking. There were only three sites where results were above the agriculture recommendations: Colusa Drain above Knight's Landing (SC of 757 umhos/cm during the July sampling event), Mokelumne River at New Hope Road (pH at 8.62 during the July sampling event and 8.78 during the December sampling event), and Harding Drain at Carpenter Road (SC above 900 umhos/cm during all sampling events). All results met the turbidity aquatic life objective, while results for water temperature and DO were outside the guidelines and objectives at half of the sites during specific times of year. When comparing *E. coli* concentrations to the Basin Plan objective for fecal coliform (of which *E. coli* is a subset) and USEPA recreational guidelines, about 12% and 35% of water samples from the Sacramento River basin exceeded the Basin Plan objectives and EPA designated beach guideline, respectively. Similarly, about 18% and 24% of water samples from the San Joaquin River basin exceeded the Basin Plan objectives and EPA designated beach guideline, respectively. These exceedances were not associated

with season, the site's land use designation, or detecting human fecal *Bacteroidales*. Instead, the presence of cow fecal *Bacteroidales* in the water sample was significantly associated with exceeding either standard, such that the odds of exceeding either standard was 30 to 40 times larger when cow fecal *Bacteroidales* were detected compared to water samples where no such fecal sources were detected.

Based on the findings of the occurrence from this study, we would recommend:

- Increasing the number of samples per site.
- Conducting future studies to address more intensive spatial and temporal sampling at the sites mentioned above,
- Improving methodology for Microbial Source Tracking (MST) and bacterial viability analyses. Bacteria viability can be assessed by reverse transcription PCR (RT-PCR) and the MST can be determined by combination of DNA fingerprinting and pulsed-field gel electrophoresis (PFGE).
- Sampling larger volumes of water for detecting *Salmonella* and *E. coli* O157:H7 for improved detection of pathogenic bacteria and perhaps limiting the *Bacteroidales* assay to just those samples that exhibit high levels of either indicator bacteria or pathogenic microorganisms.
- Conducting expanded studies, to include:
 - Epidemiology studies for *Salmonella* and *E. coli* O157 to determine feasibility for developing water quality objectives
 - Additional molecular targets, such as *E. coli* stx1, stx 2, and rfbE to better understand the virulence and toxins that bacteria produced by *E. coli* O157
 - Examination of associations of land use to the occurrence of pathogenic bacteria in watersheds.

2.0 INTRODUCTION

Previous studies conducted by Central Valley Regional Water Quality Control Board (Central Valley Water Board) programs such as the Surface Water Ambient Monitoring Program (SWAMP) and Irrigated Lands Regulatory Program (ILRP), have documented levels of *E. coli* that were elevated above the recreation objective in the Central Valley Water Board's Basin Plan for fecal coliform of 400 MPN/100 ml and USEPA's *E. coli* designated beach guideline of 235 MPN throughout the Central Valley.

While the Central Valley Water Board Water Quality Control Plan (Basin Plan) uses fecal coliform, *E. coli* has been used as an indicator of potential pathogen presence by these programs because it is a subset of fecal coliform. Also, *E. coli* is specific to warm blooded mammals, whereas fecal coliform detects not only *E. coli*, but also *Klebsiella* (which is common in both the environment and human sources) and *Citrobacter* (commonly found in mammals, birds, reptiles, and amphibians).

The Central Valley Bacteria Source Identification Study Project was initiated and contracted between the Central Valley Regional Water Quality Control Board and University of California at Davis to investigate the occurrence and source of pathogenic

bacteria in waters from the Sacramento River and San Joaquin River Basins. The objective of this study was to investigate the seasonal occurrence and evaluate the potential sources of pathogenic bacteria in watersheds draining to the two rivers.

Between May 2009 and December 2009, four sampling events were conducted. Each sampling event represented a different season: May represented late spring runoff, July represented irrigation, October represented dry, and December represented winter storm runoff. Field parameters included specific conductance, water temperature, dissolved oxygen, pH, turbidity, and photo documentation. Laboratory analysis included *E. coli*, *E. coli* O157:H7, *Salmonella*, and *Bacteroidales*.

3.0 BACKGROUND

The Sacramento River and San Joaquin River Basins cover about one fourth of the total area of the State of California and over 30 percent of the State's irrigable land. The Sacramento River basin is approximately 27,000 square miles and covers 17 percent of California's land. The San Joaquin River basin covers 17,720 square miles. These watersheds consist of two major valleys, the Sacramento Valley to the north and the San Joaquin Valley to the south. These valleys are bounded by several mountain ranges: the Coast Range to the west, the Cascade and Klamath Ranges to the north, and the Sierra Nevada to the east. Both basins provide a myriad of uses from their headwaters to discharge into the Sacramento-San Joaquin Delta, including timber production, grazing, recreation, fish habitat, drinking water supply and especially along the valley floor, agriculture. Combined, the two basins represent approximately 45% of the irrigated acreage in California.

The Sacramento watershed drains from Northern California near the Oregon border to the Delta, where it joins the San Joaquin River and San Francisco Bay.

The Sacramento River is the largest river in the watershed, with an annual average stream flow volume of 22 million acre-feet. The river is also the longest in the State, extending over 400 miles. Major tributaries to the Sacramento River include the Feather, Yuba, American, and Pit Rivers. The main stem of the Sacramento River and most of its major tributaries has been developed for water storage, flood control, and power generation.

The San Joaquin watershed originates in the southern Sierra Nevada within Madera County, and flows north approximately 300 miles to the Delta. The San Joaquin River is the principal drainage artery of the San Joaquin Valley. Average annual surface runoff for the watershed is about 1.6 million acre-feet. Major tributaries to the San Joaquin River include the Cosumnes, Mokelumne, Calaveras, Stanislaus, Tuolumne, and Merced Rivers, which primarily carry snowmelt. Flows from the west side of the river basin are dominated by agricultural return flows since west side streams are ephemeral and their downstream channels are used to transport agricultural return flows to the main channel.

The Surface Water Ambient Monitoring Program (SWAMP) and Irrigated Lands Regulatory Program (ILRP) of the Central Valley Water Board assess overall surface and agricultural water quality, respectively. During initial monitoring surveys conducted by SWAMP and ILRP, elevated concentrations of *E. coli* were detected at numerous locations throughout both basins. Some sites exceeded the USEPA designated beach guideline of 235 MPN/100ml during every sampling event, while at other sites, elevated concentrations appeared associated with flushing rainfall events.

Waterborne outbreaks of disease caused by microbial pathogen infection have been increasingly of concern to public health. Contamination of surface waters by pathogenic bacteria in California continues to impact the many beneficial uses including the sources of drinking water for municipal utilities in the watershed. Recent *E. coli* O157 and *Salmonella* outbreaks between 1996 and 2008 brought attention to water supply systems and management practices used in raising crops (see Table 1). Long-term reduction of pathogenic bacteria contamination requires an integrated approach that combines pathogen monitoring, microbial source tracking, and monitoring protocols that can detect trends in recovery or degradation of microbial water quality.

Table 1. Reports of investigations published by the California Department of Public Health to determine risk factors of bacterial contamination related to surface water that lead to bacteria outbreaks, 1996 - 2008

Report Title	Year of Publication	Risk Factor
Investigation of an <i>E. coli</i> O157:H7 Outbreak Linked to Fancy Cutt Farms	1996	No conclusive finding
Environmental Investigation of <i>Salmonella</i> Enteritidis, Phage Type 30 Outbreak Associated with Consumption of Raw Almonds	2001	Application of primary or secondary treated sewage effluent
<i>E. coli</i> O157:H7 Illnesses in Washington – July, 2002	2002	No conclusive finding
Report of Investigation of <i>E. coli</i> Outbreak at San Mateo County Retirement Facility in October 2003	2004	Flood irrigation water
Investigation of Pre-washed Mixed Bagged Salad Following an Outbreak of <i>E. coli</i> O157:H7 in San Diego and Orange County	2004	Irrigation water, drainage ditch flooding
Environmental Investigation of <i>Escherichia coli</i> O157:H7 Outbreak Associated with Taco Cell Restaurants in Northeastern States.	2007	No conclusive finding
Investigation of an <i>Escherichia coli</i> O157:H7 Outbreak Associated with Dole Pre-packaged Spinach	2007	Cattle feces, wild pig feces, soil, and river water samples
Investigation of the Taco John's <i>Escherichia coli</i> O157:H7 Outbreak Associated with Iceberg Lettuce.	2008	Lettuce growing regions in California's Central Coast and Central Valley, specifically, potential of microbial cross-

Report Title	Year of Publication	Risk Factor
		contamination between growing fields of lettuce and nearby dairies.

The Central Valley includes areas with populations ranging from under 100 in unincorporated areas to over 1 million in the Sacramento urban area. Water use is highest for agricultural and urban uses. Monitoring the seasonal concentrations of pathogenic bacteria and determining the source of contamination are some the critical actions needed to protect water quality and public health in Central Valley and the rest of California.

4.0 MONITORING OVERVIEW

This project investigated the occurrence and source of pathogenic bacteria in waters of the Sacramento River and San Joaquin basins. The detailed objectives of this project were:

- Evaluate seasonal bacteria concentrations and trends in selected water bodies within the Central Valley of California,
- Determine whether *E. coli* O157:H7 and *Salmonella* are present at any time at the sites being evaluated,
- Evaluate potential sources of fecal contamination and group potential sources to human, cattle, or other animals,
- Document the presence of source identifier DNA in viable vs. non-viable *Bacteroidales* cells in relation to the presence of *E. coli*, *E. coli* O157:H7, and *Salmonella*,
- Compare reported concentrations to appropriate water quality objectives and guidelines, including the Central Valley Regional Board Basin Plan (Basin Plan, 2007) and the USEPA Bacterial Water Quality Standards for Recreational Waters guidelines (USEPA Standards, 2003)
- Make recommendations for future bacteria source identification studies.

Based on the SWAMP's template and guidance, UC Davis researchers developed a Monitoring Plan (MP) and a Quality Assurance Project Plan (QAPP) prior to sampling. To address the objective of this project, the field measurement parameters selected included water temperature, dissolved oxygen, pH, specific conductivity, and photo monitoring; and parameters for laboratory analysis include *E. coli*, *E. coli* O157:H7, *Salmonella*, and *Bacteroidales*. All samples were collected as grab samples. UC Davis was responsible for providing sample containers, conducting laboratory analysis of *E. coli* O157:H7, *Salmonella*, and *Bacteroidales*, including viability data, managing data, and reporting to the Central Valley Water Board. The Central Valley Water Board was responsible for sampling, field measurements and observations, delivery of samples to analytical laboratories at UC Davis, and conducting laboratory analysis of *E. coli*.

Twelve sites were selected for sampling in the Sacramento River and San Joaquin River basins (Table 2) based on data from ILRP and SWAMP's previous monitoring

projects. For all sites, safety and all-weather access were priorities for sampling activities. Sampling locations were distributed throughout the Sacramento and San Joaquin River Watersheds. Locations were targeted to represent different, sometimes multiple, land uses to include integrators (2 sites), irrigated agriculture (5 sites), concentrated animal feeding operations (1 sites), recreation (5 sites), and community development (8 sites) in upper and lower watershed areas. Sampling events were distributed to capture changes in water quality over four major seasons (spring runoff, irrigation, dry and winter runoff). Table 3 summarizes nearby land uses, drainage areas, and communities which may potentially influence results at each of the sites.

Sampling events were scheduled to allow for evaluation of results across seasons. The late start to the beginning of sampling resulted in the first event representing late spring runoff. The remaining events represented irrigation, dry, and winter storm seasons.

Table 2. Summary of sampling site locations and historic *E. coli* ranges used to evaluate sites for inclusion in this study

Map ID	Station number	Site description	Local land use	Historical Ranges (MPN/100mL)	Latitude	Longitude
Sacramento Watershed						
1	A0275890 (DWR SWCMP)	Sacramento River below Red Bluff	A/E	980	40.1534	-122.1993
2	A0332000 (DWR SWCMP)	Elder Creek at Gerber	B/D	649	40.0509	-122.1666
4	A0294710 (DWR SWCMP) 520CLSAKL (SWAMP ID)	Colusa Basin Drain above Knights Landing	B	870	38.8121	-121.7741
6	531PLA900 (SWAMP ID)	Dry Creek/ Cirby Confluence	D/E	210 - >2420	38.7335	-121.2885
7	544SAC007 (SWAMP ID)	American River at Discovery Park	D/E	187 - 1414	38.6017	-121.5027
San Joaquin Watershed						
9	AMA002 (SWAMP ID)	Sutter Creek at Hwy 49	D/E	<1 - >2420	38.3926	-120.8013
10	SAC002 (SWAMP ID)	Mokelumne River at New Hope Road	A/E	23 - >2420	38.2361	-121.4189
11	SJC515 (SWAMP ID)	Bear Creek at Lower Sacramento Road*	B/D	15 - >2420	38.0428	-121.3214
12	TUO208 (SWAMP ID)	Woods Creek at Mother Lode Fairgrounds*	D	84 - 1553	37.9778	-120.3903

Map ID	Station number	Site description	Local land use	Historical Ranges (MPN/100mL)	Latitude	Longitude
13	535XLTABR (ILRP ID)	Lone Tree Creek at Brennan Rd*	B/D	>1600	37.8255	-121.0159
15	535XDCAWR (ILRP ID)	Dry Creek at La Loma Road	C	8 - >1600	37.6602	-120.8743
16	STC501 (SWAMP ID)	Harding Drain at Carpenter Road*	B/D	<1 - >2420	37.4644	-121.0303

A – Integrator Site

B – Irrigated Agriculture

C – Confined Animal Feeding Operation

D – Community Development

E – Recreation

DWR SWCMP = Department of Water Resources Surface Water

SWAMP = Surface Water Ambient Monitoring Program

ILRP = Irrigated Lands Regulatory Program

Historic Ranges = From monitoring conducted by the SWAMP and ILRP programs from 2001-2009

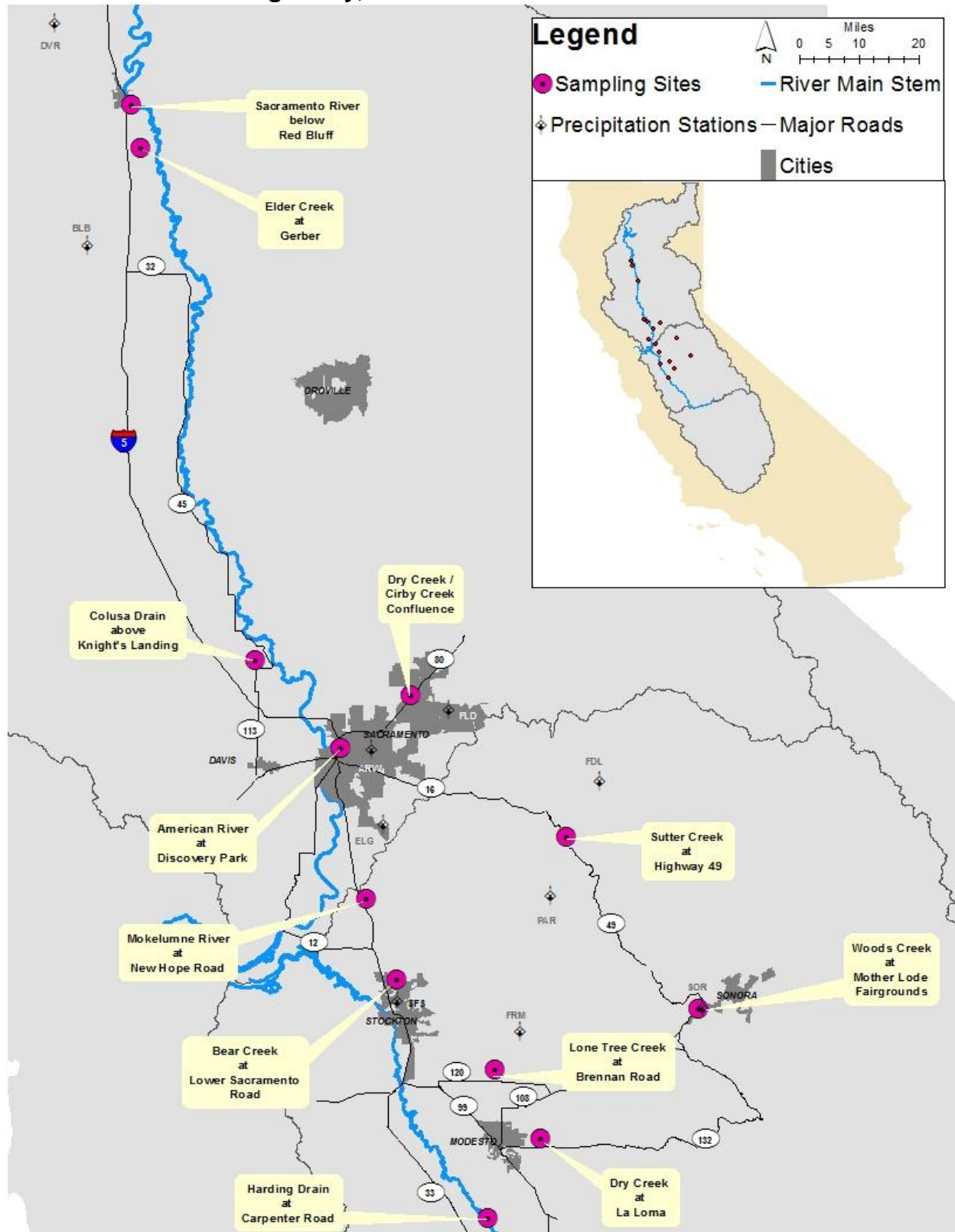
Table 3. Summary of sampling site land uses, represented drainage areas, and nearby communities

Site Description	Local Land Use (1, 2)	DWR Land Use 1 Mile upstream	Total Drainage (where available)	Nearest Upstream Community (2)	Population (3)
Sacramento River Watersheds					
Sacramento River Below Red Bluff	Within recreation area boat launch; Downstream of diversion dam (~200 ft.); adjacent to urban and privately owned farmland	Deciduous Fruits and Nuts, Grains and Hay Crops, Idle, Urban Landscape, Native Vegetation, Water Surface, Riparian Vegetation, Industrial, Vacant, Residential (1999)		Red Bluff	13,726
Elder Creek at Gerber	Within Crop Agriculture	Pasture, Field Crops, Grain and Hay Crops, Semiagricultural & Incidental to Agriculture, Native Vegetation, Idle, Residential, Riparian Vegetation, Barren and Wasteland, Vacant, Water Surface (1999)	142 square miles (1)	NA	
Colusa Basin Drain above Knights Landing	Within Crop Agriculture	Field Crops, Water Surface, Native Vegetation, Rice, Truck, Nursery and Berry Crops, Semiagricultural &	1,562 square miles (1)	NA	

Site Description	Local Land Use (1, 2)	DWR Land Use 1 Mile upstream	Total Drainage (where available)	Nearest Upstream Community (2)	Population (3)
		Incidental to Agriculture (1997)			
Dry Creek/Cirby Confluence	Within Urban, Park	Urban, Riparian Vegetation, Vacant (1994)	101 square miles (1)	Auburn	13,489
American River at Discovery Park	Within Urban, Park, Beach	Urban Landscape, Riparian Vegetation (2000)		Sacramento	481,563
				Rancho Cordova	61,747
				Folsom	71,051
				Citrus Heights	87,615
San Joaquin River Watersheds					
Sutter Creek at Highway 49	Within Park, rural community	Vacant, Urban, Native Vegetation, Vineyards, Commercial, Residential, Water Surface (1997)		Sutter Creek	2,666
Mokelumne River at New Hope Road	Downstream of agriculture, rural	Field crops, riparian vegetation, Truck, nursery, and berry crops (1996, 2000)		Lodi	63,164
Bear Creek at Lower Sacramento Road	Agriculture	Truck, nursery, and berry crops, pasture (1996)			
Woods/Sonora Creeks at Mother Lode Fairgrounds	Within rural community, downstream from rangeland	Native Vegetation, Urban, Urban Landscape, Commercial, Vacant (2004)	29 square miles (1)	Sonora	4,645
Lone Tree Creek at Brennan Road	Within agriculture; Downstream of rural	Pasture, Rice, Semi-agricultural & Incidental Agriculture (1996, 2004)		Escalon	7,185
Dry Creek at La Loma Road	Within Urban, Park	Urban, Water Surface, Vacant, Native Vegetation, Commercial, Urban Landscape (2004)		Modesto	209,574
Harding Drain at Carpenter Road	Within agriculture; Downstream of urban, including discharge from Turlock WWTP	Water Surface, Pasture, Native Vegetation, Industrial, Semiagricultural & Incidental Agriculture, Field Crops (2004)	6.64 square miles (1)	Modesto	209,574

Sources: 1) Site Descriptions, CVRWQCB; (2) Google Maps; (3) State of California, Department of Finance, E-1 Population Estimates for Cities, Counties and the State with Annual Percent Change — January 1, 2009 and 2010. Sacramento, California, May 2010

Figure 1. Sample site locations for the Central Valley Bacterial Source Identification Screening Study, 2009



Study Limitations and Observations

The scope of this study was limited in large part by contractual requirements and existing knowledge.

The budget limited subcontracting samples to approximately sixty each of *E. coli* O157, *Bacteroidales*, and bacteroidale viability samples through SWAMP. Analysis of *Salmonella* samples was funded through the Atwill laboratory. One sample of each constituent was collected at each site for each of the seasons.

The sample collection volume was the standard size for assessments using each of the constituents included in this study. The decision to use these volumes (100ml for total coliform and *E. coli*, and 1 liter for *E. coli* O157:H7, *Salmonella*, and *Bacteroidales*/viability) was based on these standards.

No portion of the study could be started prior to contract execution. The result of this was that the time allowed from initiation of drafting a Monitoring Plan and Quality Assurance Project Plan and end of the project term was 13 months, starting in February 2009.

Administrative requirements delayed the first sampling event to May, the end of the spring runoff. The irrigation season was characterized in a sampling event in July, and the dry season was characterized in a sampling event in October, although an early rain event occurred during this event. The final sampling event occurred in December in an attempt to characterize winter runoff.

Connections between sources of bacteria identified in the *Bacteroidales* classifications, other pathogens, field data and land use are limited to the data collected during this study.

Water quality objectives, guidelines, and/or recommendations do not exist for all constituents. Table 13 identifies the existing objectives, criteria, guidelines and recommendations which will be used to address the study objective to compare data to appropriate water quality objectives and guidelines. Additionally, while the Basin Plan provides an objective for fecal coliform, there is none for *E. coli*. Regardless, *E. coli* was used in this study because *E. coli* is a subset of fecal coliform. The use of *E. coli* allowed both a conservative evaluation against the Basin Plan water quality objective as well as a comparison to USEPA guidelines for various levels of contact recreation.

Land use characterization was limited to uses within sight of the sampling location. A full description of land uses and the area represented by each site would require work outside the scope of this project.

Variability due to the unique characteristics at each site which were not identified may also have influenced results.

Weather conditions and other seasonal patterns may have also influenced results.

- A rain event occurred during the dry season sampling event in October.
- Sutter Creek during the irrigation and dry season sampling events had no or limited flow. Also, during the dry season sampling event, although there was flow, it appeared that it could have been groundwater seepage.
- Flow at Elder Creek is generally intermittent with a highly fluctuating flow regime and is normally dry from July to November. We were not aware of this until sampling had already been conducted.
- Bear Creek at Lower Sacramento River site during the irrigation season sampling event: there were a noticeable amount of dead crawdads and fish. A visitor at the site at the time of the sampling event had commented that two days prior water at the site had been clear and lots of live critters were present.
- Colusa Drain at Knight's Landing during the Irrigation season sampling event: drainage approximately 25 yards upstream of the site was noted along with a smell comparable to sewage.
- American River at Discovery Park during the Irrigation season sampling event: the water stage was higher than during the spring runoff sampling event.
- Dry Creek at La Loma: On 21 May 2009, there was a sewage spill from the Modesto Collection System, potentially affecting the spring samples collected at this site.

The method used to analyze *Bacteroidales* and viability has been used in studies that were peer reviewed. However, the method is still developing. Complications occurred that raise questions regarding the methodology and lab errors. Discussions in this study include all data unless specified and questionable results are identified.

5.0 QUALITY ASSURANCE, QUALITY CONTROL, and ANALYSIS METHODS

The Measurement Quality Objectives (MQOs) are summarized in Table 4. All procedures of this study were conducted in accordance with the Quality Assurance Project Plan (QAPP) and Monitoring Plan (MP) developed for this project, which can be found at

http://www.waterboards.ca.gov/centralvalley/water_issues/water_quality_studies/surface_water_ambient_monitoring/swamp_regionwide_activities/index.shtml. The QAPP is based on the 2008 SWAMP Quality Assurance Management Plan for the State of California's Surface Water Ambient Monitoring Program and the Regional Board's San Joaquin River Procedures Manual, Appendix F Bacteria Monitoring. All data presented met MQO's specified for the project except for bacteroidale viability, as detailed in the *Bacteroidales* and Viability section that follows.

Field Parameters

Field parameters included water temperature, dissolved oxygen, pH, specific conductivity, and turbidity. An YSI 600XLM multiparameter water quality monitor was used to collect data for dissolved oxygen, electrical conductivity, pH and water temperature. A Hach 2100P turbidimeter was used for field measurements of turbidity.

Both pieces of equipment were calibrated at the beginning of each sampling event and calibration checks were performed. Acceptable ranges were determined by the manufacturers, and summarized in the method performance criteria section of the QAPP.

All equipment met calibration standards for all sampling events.

Laboratory Analytical Parameters

Blind field replicates and lab splits were collected for 5% of the samples collected. Each field crew collected an *E. coli* and total coliform field blank and one set of field blanks was collected for each sampling run for *E. coli* O157:H7, *Salmonella*, and *Bacteroidales*. Grab water samples were bottled as appropriate and held at 4°C after collection and during transportation. Chain-of-custody forms were maintained for all sampling events and for all samples.

Replicate and split samples met the Method Quality Objectives specified in Table 4 with the exceptions of blanks testing positive. During the July sampling event, blanks for *Salmonella* and *E. coli* O157:H7 tested positive. The water used for these blanks came from the Central Valley Water Board lab. This water had been processed through the Central Valley Water Board lab's deionized water machine, but had not been sterilized. During the October sampling event, the Universal *Bacteroidales* were found in the field blank. The field blank may have gotten switched with another sample since results at one of the sites indicated no *Bacteroidales* were present. And during the October sampling event, the total coliform travel blank tested positive, but the lab blank was negative. It is unclear what the potential cause of this result was.

E. coli

E. coli was analyzed at the in-house laboratory of the Central Valley Water Board, using the IDEXX Colilert® QuantiTray system.

***E. coli* O157:H7**

Water samples were analyzed for detection of the presence/absence of *E. coli* O157:H7 by the Atwill Laboratory using a Qualitative Enrichment-IMS method. For each sample 500 ml of water were filtered at the laboratory within 24 hours after collection. Filter membranes were enriched in Tryptic Soy Broth followed by Immuno-magnetic separation (IMS) previously described by Paton and Paton (2003). This IMS procedure having been intensively applied in our laboratory for detection *E. coli* O157 from samples with different matrix (e.g. feces, soils, tissue, plants, water). Because the IMS procedure extracts bacteria from the enrichment broth, it was not anticipated that water turbidity impacts the IMS. Rainbow agar plate and CT-SMAC II agar plate were used for isolation of *E. coli* O157. Specific Polymerase Chain Reaction (PCR) was performed on positive samples for O157 serogroup and H7 determination.

Salmonella

The method for *Salmonella* analysis followed the FDA Bacteriological Analytical Manual, Chapter 5, which can be found at <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm> and the article Comparative study of different methods for detection and enumeration of *Salmonella* spp. in natural waters, by Moiggo, Borrego, and Romero.

Water samples were analyzed for the presence/absence and concentration of *Salmonella* spp. by the Atwill Laboratory. An Enrichment-MPN (most probable number) method was used for detection and estimation of concentrations of *Salmonella* in waters. A regime of 200 ml × 4, 20 ml × 4, and 2 ml × 4 of water were filtered at the laboratory within 24 hours after collection. Filtration membranes were enriched in Buffered Peptone Water (BPW) and Rappaport-Vassiliadis (RV) buffer followed by isolation on Xylose Lysine Deoxycholate (XLD) agar plates. Presumptive positive *Salmonella* on XLD agar plates were confirmed by biochemical tests. Concentrations of *Salmonella* spp. in waters were calculated as MPN/100ml of water using MPN calculator software. For the May 2009 sampling event, *Salmonella* was detected in Field Blank water. The problem was corrected by reviewing and enforcing sterile procedures during sampling and transportation and by using autoclaved DI water as blanks. This problem did not occur again during the remainder of the project.

***Bacteroidales* and viability**

At the Wuertz Laboratory, using qualitative methods and quantitative models developed by the laboratory, sources of fecal contamination were grouped to human, bovine, dog and universal (all warm blood animals) as determined by host-specific *Bacteroidales* qPCR assays and analyzed for viability (alive versus dead cells).

The order *Bacteroidales* includes four families of environmental bacteria (*Bacteroidaceae*, *Rikenellaceae*, *Porphyromonadaceae*, and *Prevotellaceae*), which are all strictly anaerobic bacteria found in intestines of warm blooded animals. Thus, they represent an excellent alternative to current fecal indicators as their survival is unlikely outside of their hosts and their detection indicates relative recent fecal contamination. Using quantitative real time PCR (qPCR), this library-independent method additionally allows the differentiation of different sources in form of host-specific assays for human, bovine, and dog specific *Bacteroidales* based on selective quantification of unique gene fragments.

Besides being host specific, the use of host specific molecular assays provides the additional advantage compared to culture based methods, like the enumeration of common fecal indicator bacteria (*E. coli*, total coliforms, etc.), in that nucleic acid extracts of samples can be safely stored over a longer period of time and reanalyzed using novel or improved assays. Therefore, round-robin tests on the same extracts involving multiple laboratories across the US are possible. Due to the time limitations,

such tests are only quite locally possible for culture based methods. Also, one common problem of culture based methods is that target organism concentrations can be too high and cultivation leads to occurrence of right censored data with high limits (e.g. > 100,000 MPN/mL). Thus, concentrations have to be estimated prior to cultivation to prepare appropriate dilutions. Since samples can only be analyzed once, a possible equipment failure (incubator not working) can cause the loss of data for a complete sample set.

Disadvantages of *Bacteroidales* Microbial Source Tracking (MST) methods include the lack of strict regulation for all steps of the analysis and the high number of different published host-specific assays with different specificities and sensitivities. Comparability of results with other laboratories is therefore still problematic which is clearly due to the novelty of the method.

Method:

Water samples were concentrated via dead-end filtration over 0.22 µm membranes. This process immobilizes *Bacteroidales* cells together with any other constituents or agglomerates of such larger than 0.22 µm on the membrane, which is subsequently subjected to nucleic acid extraction. These extracts are then analyzed through DNA sequence specific quantification via qPCR using specific primers and probe for each assay (Kildare et al. 2007). As a result the complete DNA either free or within intact *Bacteroidales* cells is quantified.

For the additional identification of only DNA markers from intact (viable) *Bacteroidales* cells, a method was recently developed using Propidium Monoazide (PMA) prior to qPCR analysis. This PMA-qPCR protocol, as described by Bae and Wuertz (2009), has the significant advantage over other MST methods of eliminating free DNA and thus quantifying only DNA within intact cells, here defined as viable cells. The published protocol, however, was optimized and validated for liquid samples. For this project the protocol was transferred to dead-end filtration. This means subsamples were filtered over two identical membranes from which only one was treated with PMA before nucleic acid extraction.

Unfortunately, after analyzing the samples via PMA-qPCR, we have to express doubts about the validity of the method based on the results gathered. Per our definition, concentrations from PMA-qPCR can never be higher than from qPCR. For universal *Bacteroidales*, however, 11 of 26 positive samples tested with both methods exhibited significantly higher “viable” concentrations than measured for total DNA. The same could be observed for 1 of 1 positive human-specific *Bacteroidales* sample and 2 of 6 positive cow-specific *Bacteroidales* samples. The fact that non-detect samples for total DNA were positive when analyzed via PMA-qPCR can be explained by statistic effects for low concentration samples when performing replicate filtrations. But statistically the PMA-qPCR dataset as such should exhibit lower concentrations than data for total DNA even when considering certain expected fluctuations between the two filtrations. One possible explanation can be the insufficient contact of PMA with free DNA due to the build-up of a filter cake which could not be penetrated by PMA.

While re-growth of anaerobic *Bacteroidales* outside of their animal hosts is clearly impossible in the presence of oxygen their DNA signal can be relatively persistent in the environment under certain conditions. The PMA-qPCR can limit the detection to intact cells only and thus narrow the window of what is considered “recent” even more; equal to culture based methods without the risk of included aged and re-grown cultures. Both methods (qPCR and PMA-qPCR) together provide information about how much and when fecal contamination occurred.

Quantification and Sample Limit of Detection (SLOD) (see also Kildare et al 2007 and Rajal et al 2007a)

Each 25 µL PCR reaction contained 12.5 µL of commercially available TaqMan PCR mastermix (Eurogentec, San Diego, CA, USA) with 400 nM each of forward and reverse primers and 80 nM probe for the respective TaqMan system. For all TaqMan reactions, 10 µL of the diluted gDNA sample was assayed in a final reaction volume of 25 µL. In order to suppress inhibitors, bovine serum albumin (BSA) was added to each reaction in a final concentration of 50 ng/µL, and four serial dilutions were performed to assess inhibition factors. Cycling conditions were 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C, using an ABI Prism 7000 (Applied Biosystems, Carlsbad, CA, USA). *Bacteroidales* assay primers and specificity were established previously (Kildare et al 2007). Concentrations and sample limits of detection (SLOD) were analyzed according to Rajal et al. (2007a). Standard curves were established by measuring plasmid concentrations as obtained through cloning of each individual target sequence in *E. coli*. These SLODs are individual limits of detection for each sample and account for concentration factors, recoveries and qPCR inhibition to help evaluating non-detects. The SLOD in gene copies/mL (gc/mL) values are calculated as follows:

$$SLOD = \left(\frac{1000 \cdot ALOD \cdot I}{C_{filtr} \cdot C_{extr} \cdot R} \right)$$

Where SLOD (gc/uL) is the assay limit of detection for the applied assay and specific conditions, C indicates concentration factors for filtration (C_{filtr}) or nucleic acid extraction (C_{extr}). The overall recovery proportion, R, is assessed by measurement of known spike doses of a bacterial surrogate, *Acinetobacter baylyi* strain ADP1, previously referenced as *Acinetobacter* sp. strain ADP1.

The parameters measured accuracy, precision, sensitivity and reporting limits, as well the completeness of project is listed in Table 4.

Table 4. Measurement quality objectives

Group	Parameter	Accuracy	Precision	Recovery/ Sensitivity	Target Reporting Limit	Calibration	Calibration Interval	Completeness
Field Testing (YSI 600XLM)	Dissolved Oxygen	±0.5 mg/L	0.01 mg/L	NA	0.01 mg/L	Saturated air	Each sampling event	90%
Field Testing (YSI 600XLM)	pH	±0.2 unit	0.01 unit	NA	NA	Buffer solutions pH 4, 7, and 10	Each sampling event	90%
Field Testing (YSI 600XLM)	Electrical Conductivity	±0.5% of reading + 0.001 mS/cm	0.001 to 0.1 mS/cm (range dependent)	NA	0.001 µS/cm	1000 uS/cm standard	Each sampling event	90%
Field Testing (YSI 600XLM)	Water temperature	± 0.15°C	0.01°C	NA	NA	Not required	Not required	90%
Field Testing (YSI 6920)	Turbidity	±2% of reading or 0.3 NTU, whichever is greater	0.1 NTU	NA	0 to 1,000NTU	StablCal 2100P	Each sampling event	90%
Laboratory Analysis (Central Valley Regional Board)	<i>E. coli</i>	Lab duplicate within 95% CI Stated by Idexx	Lab duplicate, blind field duplicate within 25% RPD (na if native concentration of either sample <RL)	Laboratory blank, field blank <1	1	NA	NA	90%
Laboratory Analysis (Atwill Lab)	<i>E. coli</i> O157:H7	Positive and negative standards test ≥90% accurate	Duplicate samples ≥80% concordant	Distinguish 0 from ≥1 MPN*	≥1 cfu per liter	NA	NA	80%
Laboratory Analysis (Atwill Lab)	<i>Salmonella</i>	Positive and negative standards test ≥90% accurate	Duplicate samples ≥80% concordant	Distinguish 0 from ≥1 MPN	≥1 cfu per liter	NA	NA	80%
Laboratory Analysis (Wuertz Lab)	<i>Bacteroidales</i>	Positive and negative standards test ≥90% accurate	Lab duplicates are ≥80% concordant	1-4 gene copies per PCR reaction per vertebrate source	1-4 gene copies per PCR reaction per vertebrate source	NA	NA	80%
Laboratory Analysis (Wuertz Lab)	Bacteroidale viability	NA	NA	NA	NA	NA	NA	NA

6.0 RESULTS

General Overview

Precipitation and Flow: May – December 2009

The San Joaquin River and Sacramento River Indices, as described in the Water Quality Control Plan for the San Francisco Bay/Sacramento-San Joaquin Delta Estuary (SWRCB, 1995) is used to classify the water year type in the rivers based on runoff. The Indices include five classifications: wet, above normal, below normal, dry and critical, based on millions of acre-feet of unimpaired annual flow.

The water year (WY) starts 1 October and ends 31 September of the following year. Because of the time of this study, May – December 2009, portions of both WYs 2008 and 2009 are represented. The classification determination for May – September 2009 for both rivers was critical, and the classification for October – December 2009 was dry in the Sacramento River and below normal in the San Joaquin River.

A rain event occurred during the sampling for the Dry Season in October. Figure 2 illustrates incremental precipitation May through December 2009 from the precipitation stations listed in Table 6. Flow was not tracked since flow stations relevant to all but one of the sampling sites could not be identified through the California Data Exchange Center.

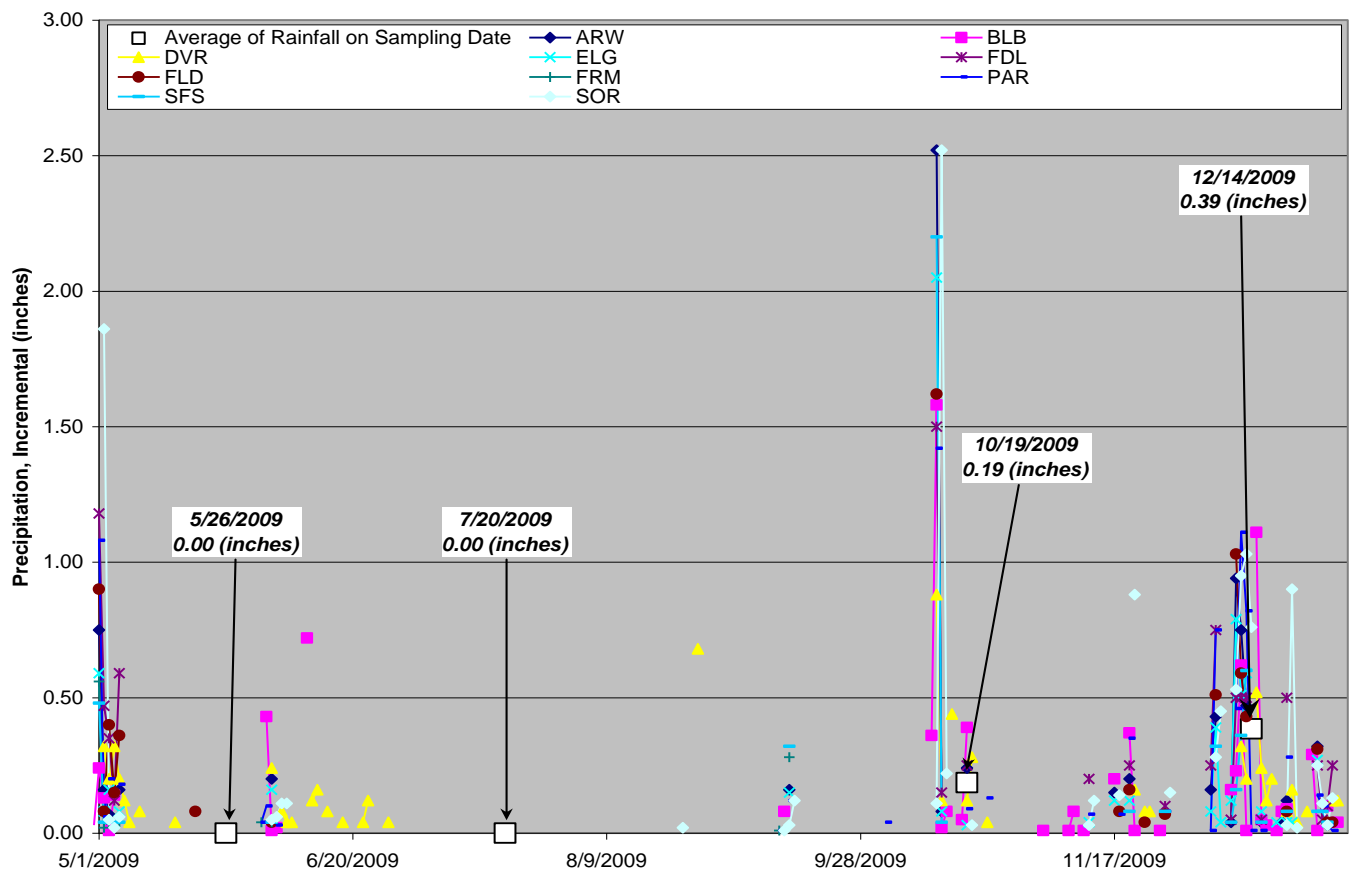
Table 5 Precipitation Stations from the California Data Exchange Center

ID	Station Name	River Basin	County	Longitude	Latitude	Elevation (feet)	Operator
DVR	Davis Ranch	Cottonwood Creek	Tehama	-122.45	40.35	550	USBR
BLB	Black Butte	Stony Creek	Tehama	-122.33	39.81	426	USACE
FLD	Folsom Dam	American River	Sacramento	-121.17	38.70	350	NWS
ARW	Arden Way	American River	Sacramento	-121.41	38.60	35	Sacramento County
ELG	Elk Grove Hatchery	Cosumnes River	Sacramento	-121.37	38.42	45	Sacramento County
FDL	Fiddletown	Cosumnes River	Amador	-120.70	38.53	2160	USBR
PAR	Pardee	Mokelumne River	Calaveras	-120.85	38.25	568	EBMUD
SFS	Stockton Fire Station	San Joaquin River	San Joaquin	-121.32	37.99	14	CA DWR
FRM	Farmington	Littlejohn Creek	San Joaquin	-120.94	37.92	180	USACE

ID	Station Name	River Basin	County	Longitude	Latitude	Elevation (feet)	Operator
SOR	Sonora RS	Tuolumne River	Tuolumne	-120.38	37.98	1749	TUD

- 1 US Bureau of Reclamation
- 2 US Army Corps of Engineers
- 3 National Weather Service
- 4 East Bay Municipal Utility District
- 5 California Department of Water Resources
- 6 Tuolumne Utility District

Figure 2 Incremental precipitation from stations reporting to the California Data Exchange Center, May - December 2009



Elder Creek at Gerber was dry during the July, October, and December sampling events, and Sutter Creek was dry during the July sampling event.

Sampling Data

Data collected during this study are summarized in Table 6, using minimum, mean, and maximum concentrations for each constituent, by site.

Overall, water temperature ranged from 9.86°C at Lone Tree Creek at Brennan to 28.14°C at Colusa Drain above Knight's Landing and the mean was 19.33°C. Water temperature was slightly higher at the Sacramento River sites (ranging from 14.67°C at Sacramento River Below Red Bluff to 28.14°C at Colusa Drain with an average of 19.37°C) than at the San Joaquin River sites (ranging from 9.86°C at Lone Tree Creek at Brennan Road to 26.58°C at Bear Creek at Lower Sacramento Road, with an average of 18.31°C).

Specific conductivity (SC) overall ranged from 44 umhos/cm at Mokelumne River at New Hope Road to 959 umhos/cm at Harding Drain at Carpenter Road. Ranges were tighter at river sites (under 50 umhos/cm) than creeks (60 to 285 umhos/cm). The overall mean specific conductivity was 286 umhos/cm. In the Sacramento watershed, SC ranged from 53 umhos/cm at American River at Discovery Park to 757 umhos/cm at Colusa Basin Drain. The mean was 251 umhos/cm. In the San Joaquin River watershed, the lowest SC was 44 umhos/cm at Mokelumne River at New Hope Road, the highest was 959 umhos/cm at Harding Drain, and the mean was 307 umhos/cm.

Dissolved oxygen (DO) concentrations ranged from 1.83 mg/l at Sutter Creek at Highway 49 to 13.14 mg/l at Sacramento River at Red Bluff. The overall mean was 7.72 mg/l. In summarizing results by watershed, DO was lower in the San Joaquin than Sacramento watershed. The minimum and maximum results in the San Joaquin River watershed were 1.83mg/l at Sutter Creek and 10.37 mg/l at Harding Drain, and the mean was 7.36 mg/l. In the Sacramento River watershed minimum DO was 3.24 mg/l at Colusa Basin Drain and the maximum was 13.14 mg/l at Sacramento River below Red Bluff. The mean was 8.29 mg/l.

The pH ranged from 6.11 at Sutter Creek to 8.62 at Mokelumne River at New Hope, and had an average of 7.48. The minimum and average pH were higher in the Sacramento River sites (6.94 and 7.67, respectively, both at American River at Discovery Park) than in the San Joaquin River sites (6.11 and 7.37, respectively), while the maximum pH was slightly higher in the San Joaquin River watershed (8.62 at Mokelumne River at New Hope Road) than in the Sacramento River watershed (8.40 at Sacramento River below Red Bluff).

Turbidity ranged from 1.00 NTU at Sutter Creek to 43.17 NTU at Colusa Basin Drain, and the average of all results was 16.47 NTU. Minimum concentrations in each of the watershed sites were nearly identical (1.04 NTU at Elder Creek in the Sacramento watershed and 1.00 at Sutter Creek). Mean concentrations were slightly higher in the San Joaquin River watershed (16.36 NTU) than Sacramento River watershed (15.69 NTU). The maximum concentration was higher in the Sacramento River watershed (43.17 NTU at Colusa Basin Drain above Knight's Landing) than in the San Joaquin River watershed (33.43 NTU at Lone Tree Creek at Brennan Road).

Total Coliform was above reporting limits (>2420 MPN/100ml) in all but three samples – two at Sacramento River below Red Bluff (1120 MPN/100 ml on 5/26/2009 and 727 MPN/100 ml on 12/14/2009) and one at Sutter Creek 1733 MPN/100 ml on 12/14/2009).

E. coli ranged from 16 MPN/100 ml at Sacramento River below Red Bluff to above reporting limits at Lone Tree Creek at Brennan Road. The overall median was 166 MPN/100ml. For all summary values (minimum, median, and maximum concentrations), results were higher in the San Joaquin River watershed (23 MPN/100 ml at Bear Creek at Lower Sacramento Road, 172 MPN/100ml, and >2420 at Lone Tree Creek at Brennan Road, respectively) than in the Sacramento River watershed (16 MPN/100 ml at Sacramento River below Red Bluff, 155 MPN/100 ml, and 816 MPN/100 ml at American River at Discovery Park, respectively).

E. coli O157:H7 was detected in only two water samples during the entire project, one positive in the Sacramento River watershed (Sacramento River below Red Bluff on 7/20/2009) and one in the San Joaquin River watershed (Lone Tree Creek at Brennan Road on 5/26/2009).

Salmonella was present in 36% of the samples – five out of 17 samples in the Sacramento River watershed and eleven out of 27 samples in the San Joaquin River watershed. The maximum concentration in both watersheds was 1.2 MPN/100ml (at Elder Creek at Gerber and Harding Drain at Carpenter Road), and the mean concentration in the Sacramento watershed (0.16 MPN/100 ml overall and 0.55 MPN/100 ml where results were above 0.0 MPN/100ml) was higher than in the San Joaquin River watershed (0.15 MPN/100ml overall and 0.39 MPN/100ml where results were above 0.0 MPN/100ml).

Universal source bacteria were detected in all sampling sites for all seasons except for one negative sample at Colusa Drain above Knight's Landing. This negative sample is questionable since the marker was found in the viability analysis sample that complimented this sample, universal *Bacteroidales* were found in all other samples, and water at this site is composed of agricultural drain water.

Human source bacteria were only detected at the Dry Creek/Cirby Creek confluence (62 gc/ml) and Woods Creek at Mother Lode fairgrounds (901-4980 gc/ml), sites identified as community development and/or recreation. Cow source bacteria were detected at Dry Creek/Cirby Creek confluence (164-219 gc/ml), Sutter Creek (76 gc/ml), Woods Creek at Mother Lode Fairgrounds (220-2096 gc/ml), Lone Tree Creek (1062 – 16054 gc/ml), Dry Creek at La Loma (164-219 gc/ml), and Harding Drain at Carpenter Road (411-2834 gc/ml). These sites were identified as irrigated agriculture, confined animal feeding operation, community development, and recreation. Dog source bacteria were detected at just one site (Lone Tree Creek at Brennan Road; 16 gc/ml), which was identified as mixed for irrigated agriculture and community development.

Using mixed effects logistic regression (site set as a group effect due to repeated sampling, presence of each land use designation as the independent variable, and presence of human or cow fecal source as the dependent variable), land use designation was not associated with the likelihood of finding human or cow fecal sources.

Bacteroidale viability analysis for comparing viable to non-viable cells resulted in questionable data, as described in the Quality Assurance and Quality Control section. Although the viability of bacteria could not be reliably determined, the ratios that resulted from the analysis are included in Table 6 to provide a record of the results. The protocol will continue to be validated for application to these types of water samples.

Summary of Results

In the Sacramento River watershed sites, overall the averages of water temperature, dissolved oxygen, specific conductance, turbidity and pH were 17-C, 8.9 mg/l, 247.4 umhos/cm, 15.7 NTU and 7.8, respectively. Overall averages for San Joaquin River watershed sites were 17.5-C, 8.0 mg/l, 303.3 umhos/cm, 16.5 NTU, and 7.3, respectively. Minimum water temperature, SC, pH, average turbidity, and universal bacteroidale; and maximum water temperature, DO, and average turbidity results were higher in the Sacramento watershed than San Joaquin. Summary results were higher in the San Joaquin River watershed than Sacramento for minimum total coliform and *E. coli* and maximum SC, pH, total coliform, *E. coli*, and *Bacteroidales*.

Table 6. Summary of results

Site Code	Site Description	Temp °C		field SC umhos /cm	DO mg/l	pH	Turbidity Average NTU	Total Coliform MPN /100ml	E. coli* MPN /100ml	E. coli O157 P/A**	Salmonella MPN /100ml	Bacteroidales							
												Universal		Human		Bovine		Dog	
												gc/ml	Via-bility Ratio	gc/ml	Via-bility Ratio	gc/ml	Via-bility Ratio	gc/ml	Via-bility Ratio
504 SACRBF	Sacramento River below Red Bluff	min	14.67	118	10.33	8.05	3.62	727	16	P	0	736	0.11	0	0.00	0	0.00	0	0.00
		mean	15.80	124	11.65	8.27	7.69	923.45	41		0.045	7497	0.27						
		max	16.72	134	13.14	8.40	10.23	>2420	184		0.18	15880	0.43	0	0.00	0	0.00	0	0.00
504 ELD99W	Elder Creek at Gerber (Hwy99W)	min	24.42	393	8.6	8.21	1.04	>2420	44	A	1.2	1022	0.67	0	0.00	0	0.00	0	0.00
		mean																	
		max																	0.00
520 CLSAKL	Colusa Drain above Knights Landing	min	18.01	472	3.24	7.41	35.97	>2420	31	A	0	1046	1.34	0	0.00	0	0.00	0	0.00
		mean	23.08	613	4.3	7.51	39.66	>2420	31		0.115	23278	1.34						
		max	28.14	757	5.16	7.66	43.17	>2420	101		0.46	67577	1.34	0	0.00	0	0.00	0	0.00
531 PLA900	Dry Creek/Cirby Confluence	min	16.02	153	7.47	7.14	6.30	>2420	155	A	0	4185	0.76	62	0.00	1303	1.10	0	0.00
		mean	19.74	165	8.2	7.32	7.94	>2420	345		0.1825	43181	1.58	62		1303	1.10		
		max	24.00	185	9.06	7.42	9.57	>2420	461		0.73	93840	2.41	62	0.00	1303	1.10	0	0.00
544 SAC007	American River at Disc Park	min	15.52	53	7.7	6.94	1.94	>2420	192	A	0	1507	0.65	0	0.00	0	0.00	0	0.00
		mean	17.17	54	8.93	7.41	2.26	>2420	428		0.045	4645	1.28						
		max	19.10	55	9.64	7.68	2.83	>2420	816		0.18	7566	1.91	0	0.00	0	0.00	0	0.00
532 AMA002	Sutter Creek at Hwy 49	min	14.09	219	1.83	6.11	1.00	1732.9	161	A	0	5339	0.20	0	0.00	76	0.00	0	0.00
		mean	15.83	319	5.09	6.66	2.55	>2420	161		0	16621	3.48			76			
		max	17.56	419	8.34	7.21	4.11	>2420	172		0	29776	6.77	0	0.00	76	0.00	0	0.00
544 SAC002	Mokelumne River at New Hope	min	15.48	44	8.57	6.60	6.15	>2420	70	A	0	1141	0.11	0	0.00	0	0.00	0	0.00
		mean	20.16	59	9.37	7.70	8.26	>2420	102		0.09	8692	1.05						
		max	25.89	87	9.79	8.62	11.33	>2420	387		0.18	22040	1.99	0	0.00	0	0.00	0	0.00
531 SJC515	Bear Creek at Lower Sac Rd	min	18.08	57	3.77	6.55	13.67	>2420	23	A	0	9493	0.19	0	0.00	0	0.00	0	0.00
		mean	22.45	118	4.90	7.09	22.00	>2420	146		0.275	22440	0.54						
		max	26.58	211	6.58	7.73	31.50	>2420	152		0.46	38525	0.90	0	0.00	0	0.00	0	0.00
536 TUO208	Woods Creek at Mother	min	14.29	378	8.26	7.03	1.33	>2420	138	A	0	714	0.39	901	1.22	220	0.00	0	0.00
		mean	16.62	407	8.72	7.69	2.73	>2420	162		0.09	23038	0.40	2446	1.22	1158	0.00		
		max	19.75	459	9.05	8.04	4.86	>2420	1414		0.18	80648	0.40	4980	1.22	2097	0.00	0	0.00
535 XLTABR	Lone Tree Creek at Brennan Road	min	9.86	120	5.75	6.93	23.47	>2420	1203	P	0	35983	1.14	0	0.00	1063	0.00	16	0.00
		mean	16.67	191	6.53	7.22	28.98	>2420	1553		0.0925	331380	1.95			6751	0.43	16	
		max	21.23	301	7.44	7.58	33.43	>2420	2420		0.37	1078312	2.76	0	0.00	16054	0.85	16	0.00
535 STC206	Dry Creek at La Loma Road	min	16.29	113	6.77	7.21	4.47	>2420	184	A	0	8821	0.43	0	0.00	164	0.00	0	0.00
		mean	20.94	174	7.19	7.48	15.37	>2420	483		0	21650	2.15			191			
		max	25.45	234	7.74	7.73	23.67	>2420	813		0	47547	3.87	0	0.00	219	0.00	0	0.00
535 STC501	Harding Drain at Carpenter Road	min	19.45	902	8.23	7.17	4.33	>2420	77	A	0	6764	0.38	0	0.00	411	1.65	0	0.00
		mean	22.23	926	9.24	7.55	7.34	>2420	172		0.46	321996	1.07			1623	1.65		
		max	25.07	959	10.37	7.76	10.10	>2420	921		1.2	1156858	1.76	0	0.00	2835	1.65	0	0.00

*E. coli median values are listed instead of mean. **P=Present, A=Absent

7.0 DISCUSSION

Objective 1: Evaluate seasonal bacteria concentrations and trends in selected water bodies within the Central Valley of California

Samples were collected in an attempt to discuss seasonal constituent patterns:

- Spring runoff, collected in May
- Irrigation, collected in July
- Dry, collected in October (during which a rain event occurred)
- Winter storm runoff collected in December

Although data is limited, some trends and anomalies could be identified.

Data was incomplete for developing seasonal trends at Sutter Creek and Elder Creek due to the sites being dry and therefore not evaluated. Photo documentation of the sites can be found in Appendix B. In the figures for this section, results for sites in the Sacramento River Watershed are connected by dashed pink lines and sites in the San Joaquin River Watershed are connected by solid blue lines.

Field parameters

Field parameter results are summarized by season in Table 7 for each site.

Table 7. Field parameter measurements by season and sampling site

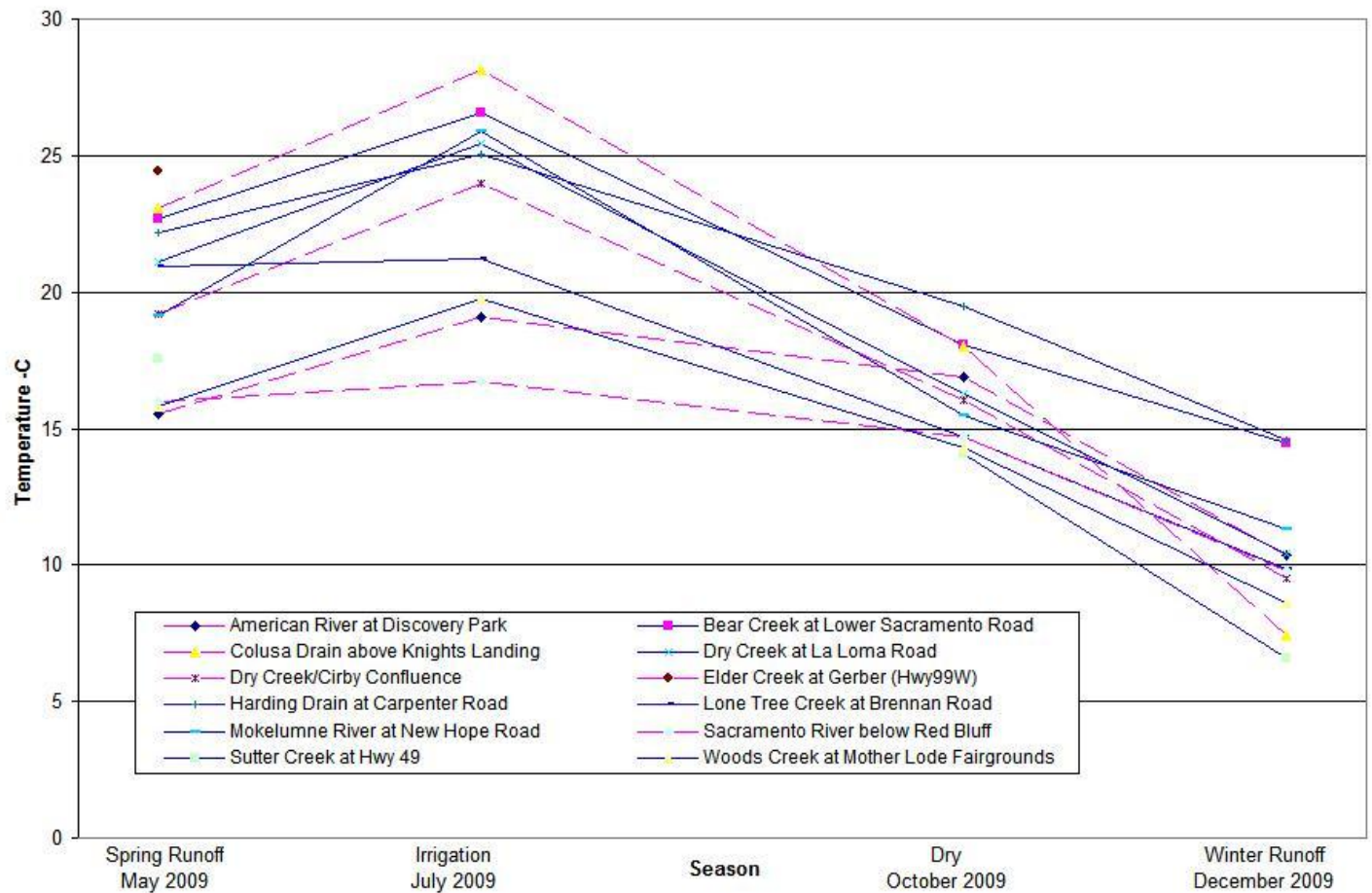
Map ID	Station number	Site description	Sampling season	Field parameters				
				Temp (°C)	DO (mg/L)	SC (umhos/cm)	Tur (NTU)	pH
Sacramento River Watersheds								
1	A0275890 (DWR SWCMP)	Sacramento River below Red Bluff	Spring runoff	16.0	11.5	118	9.2	8.4
			Irrigation	16.7	13.1	120	3.6	8.4
			Dry*	14.7	10.3	134	10.2	8.1
			Winter runoff	9.8	11.6	147	2.8	8.3
2	A0332000 (DWR SWCMP)	Elder Creek at Gerber	Spring runoff	24.4	8.6	393	1.0	8.2
			Irrigation	Dry	Dry	Dry	Dry	Dry
			Dry*	Dry	Dry	Dry	Dry	Dry
			Winter runoff	Dry	Dry	Dry	Dry	Dry
4	A0294710 (DWR SWCMP) 520CLSAKL (SWAMP ID)	Colusa Drain above Knights Landing	Spring runoff	23.1	4.5	610	43.2	7.7
			Irrigation	28.1	3.2	757	36.0	7.5
			Dry*	18.0	5.2	472	39.8	7.4
			Winter runoff	7.4	9.4	547	70.9	7.8
6	531PLA900 (SWAMP ID)	Dry Creek/ Cirby Confluence	Spring runoff	19.2	8.1	185	9.6	7.4
			Irrigation	24.0	7.5	157	6.3	7.4
			Dry*	16.0	9.1	153	8.0	7.1
			Winter runoff	9.5	11.1	191	15.0	7.8
7	544SAC007 (SWAMP ID)	American River at Discovery Park	Spring runoff	15.5	9.6	55	2.8	7.6
			Irrigation	19.1	9.4	53	2.0	7.7
			Dry*	16.9	7.7	55	2.0	6.9
			Winter runoff	10.4	11.7	58	4.4	8.4
Arithmetic mean				17.0	8.9	247	15.7	7.8
San Joaquin River Watersheds								

Map ID	Station number	Site description	Sampling season	Field parameters				
				Temp (°C)	DO (mg/L)	SC (umhos/cm)	Tur (NTU)	pH
9	AMA002 (SWAMP ID)	Sutter Creek at Hwy 49	Spring runoff	17.6	8.3	219	1.0	7.2
			Irrigation	Dry	Dry	Dry	Dry	Dry
			Dry*	14.1	1.8	419	4.1	6.1
			Winter runoff	6.6	12.7	150	4.3	6.1
10	SAC002 (SWAMP ID)	Mokelumne River at New Hope Road	Spring runoff	19.1	9.8	45	11.3	7.9
			Irrigation	26.0	8.6	87	7.3	8.6
			Dry*	15.5	9.8	44	6.2	6.6
			Winter runoff	11.3	11.6	45	4.3	8.8
11	SJC515 (SWAMP ID)	Bear Creek at Lower Sacramento Road*	Spring runoff	22.7	3.8	85	13.7	7.7
			Irrigation	26.6	6.6	57	31.5	7.0
			Dry*	18.1	4.4	211	20.8	6.6
			Winter runoff	14.4	10.5	146	131.0	7.2
12	TUO208 (SWAMP ID)	Woods Creek at Mother Lode Fairgrounds*	Spring runoff	15.8	9.1	383	2.0	8.0
			Irrigation	19.8	8.3	459	4.9	8.0
			Dry*	14.3	8.9	378	1.3	7.0
			Winter runoff	8.6	11.7	286	10.0	6.9
13	535XLTABR (ILRP ID)	Lone Tree Creek at Brennan Rd*	Spring runoff	20.9	7.4	125	31.3	7.6
			Irrigation	21.2	7.0	120	33.4	7.2
			Dry*	14.7	5.9	216	23.5	7.2
			Winter runoff	9.9	5.8	301	27.7	6.9
15	535XDCAW R (ILRP ID)	Dry Creek at La Loma Road	Spring runoff	21.1	7.7	113	23.7	7.7
			Irrigation	25.5	7.1	175	18.0	7.5
			Dry*	16.3	6.8	234	4.5	7.2
			Winter runoff	10.4	3.5	153	5.9	7.2
16	STC501 (SWAMP ID)	Harding Drain at Carpenter Road*	Spring runoff	22.2	10.4	916	4.3	7.7
			Irrigation	25.1	9.1	959	10.1	7.8
			Dry*	19.5	8.2	902	7.6	7.2
			Winter runoff	14.6	11.1	961	2.3	7.1
Arithmetic mean				17.5	8.0	303	16.5	7.3

* A rain storm occurred during this sampling event, potentially influencing water chemistry values.

Water temperature

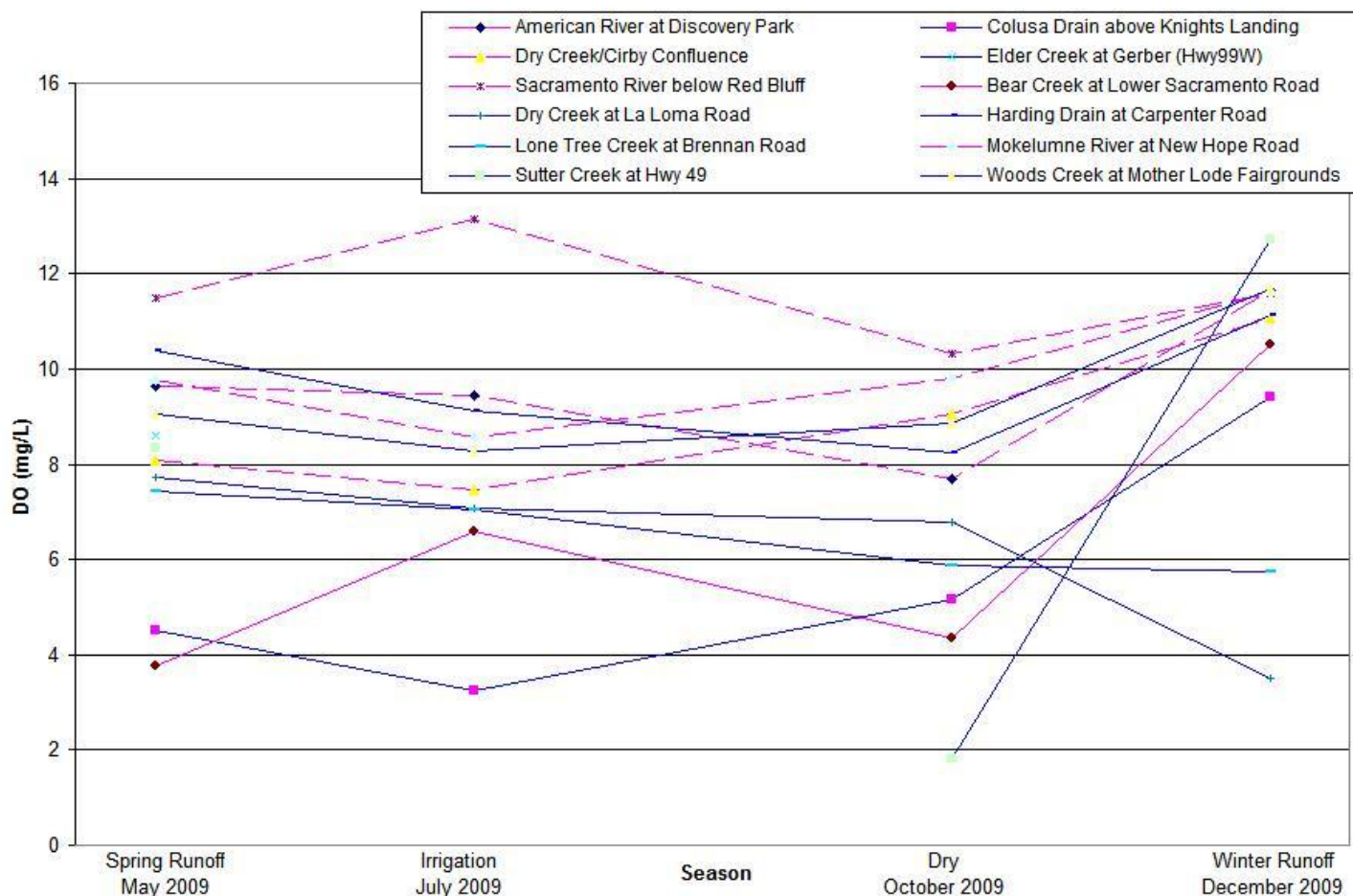
Figure 3. Seasonal trends of water temperature



Results for water temperature showed the most consistent seasonal pattern at all sites. Water temperatures increased during the irrigation season sampling event from the spring runoff sampling event by amounts ranging from less than 1 to 5 –C. Water temperatures during the dry season sampling event generally dipped below spring runoff, with temperatures dropping by 1.3 to 6.3°C and continued to drop during the winter runoff sampling event.

Dissolved Oxygen

Figure 4. Seasonal trends of Dissolved Oxygen in water



Dissolved oxygen typically follows an inverse pattern from water temperature, as was the case at most of the sites. Concentrations generally decreased during the irrigation season as compared to the spring runoff season, then followed an increasing trend through the dry and winter runoff seasons. Exceptions to this trend were at Sacramento River below Red Bluff, American River at Discovery Park, Lone Tree Creek at Brennan Road, Dry Creek at La Loma, and Bear Creek at Lower Sacramento River Road.

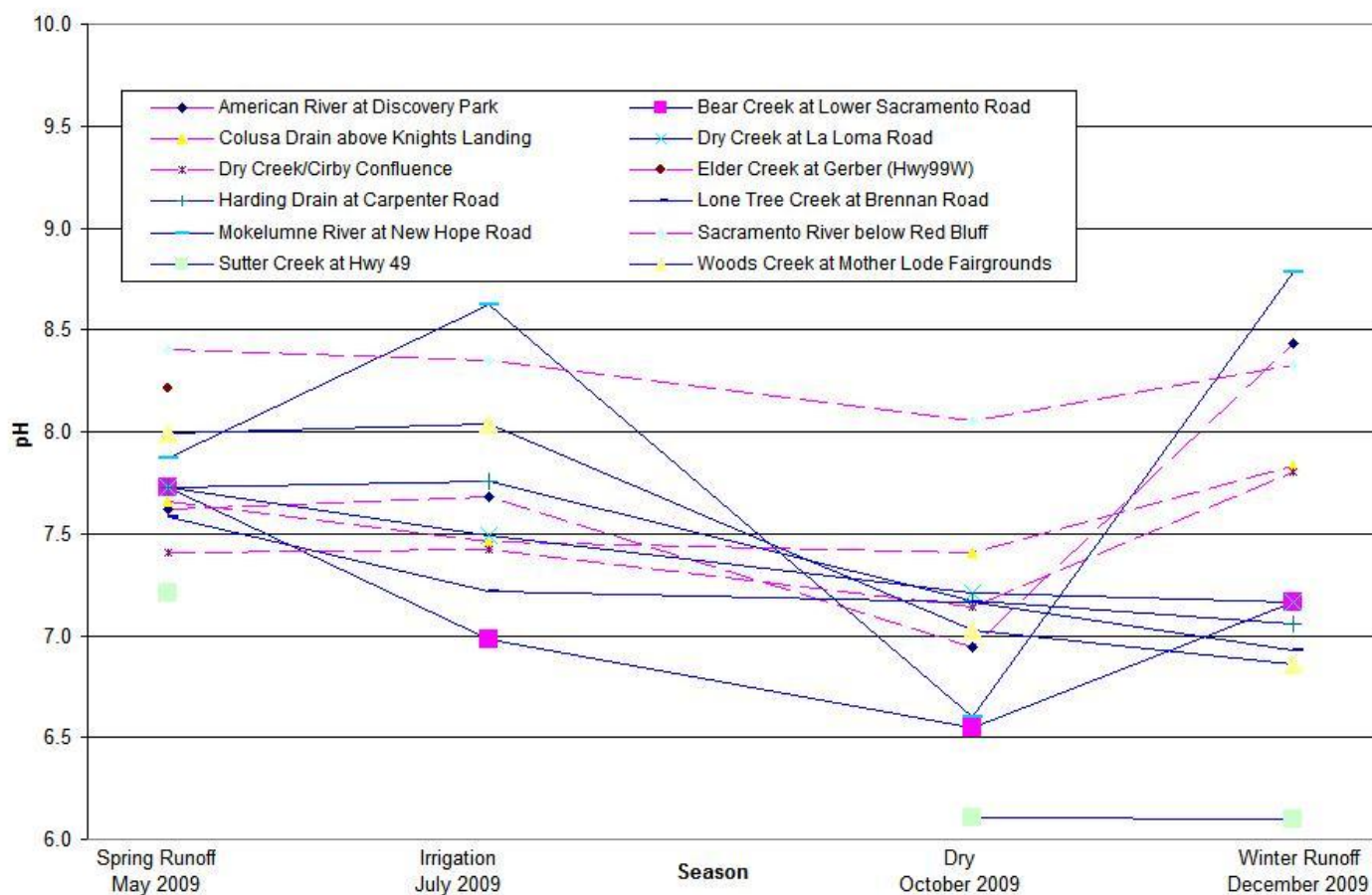
During the irrigation season sampling event at Sacramento River at Red Bluff and Bear Creek at Lower Sacramento River Road, concentrations increased rather than decreased.

At American River at Discovery Park, DO concentrations were nearly identical during the spring runoff and irrigation seasons, then dipped during the dry season and increased during the winter runoff.

Concentrations at Lone Tree Creek at Brennan Road and Dry Creek at La Loma declined throughout the year. The difference between high and low concentration at Lone Tree Creek was 1.56 mg/l, with the greatest decrease between the irrigation and dry seasons (1.15 mg/l). The difference between high and low concentrations at Dry Creek at La Loma Road was 4.24 mg/l, with the greatest decrease between the dry and winter runoff seasons (3.27 mg/l).

pH

Figure 5. Seasonal trends of water pH



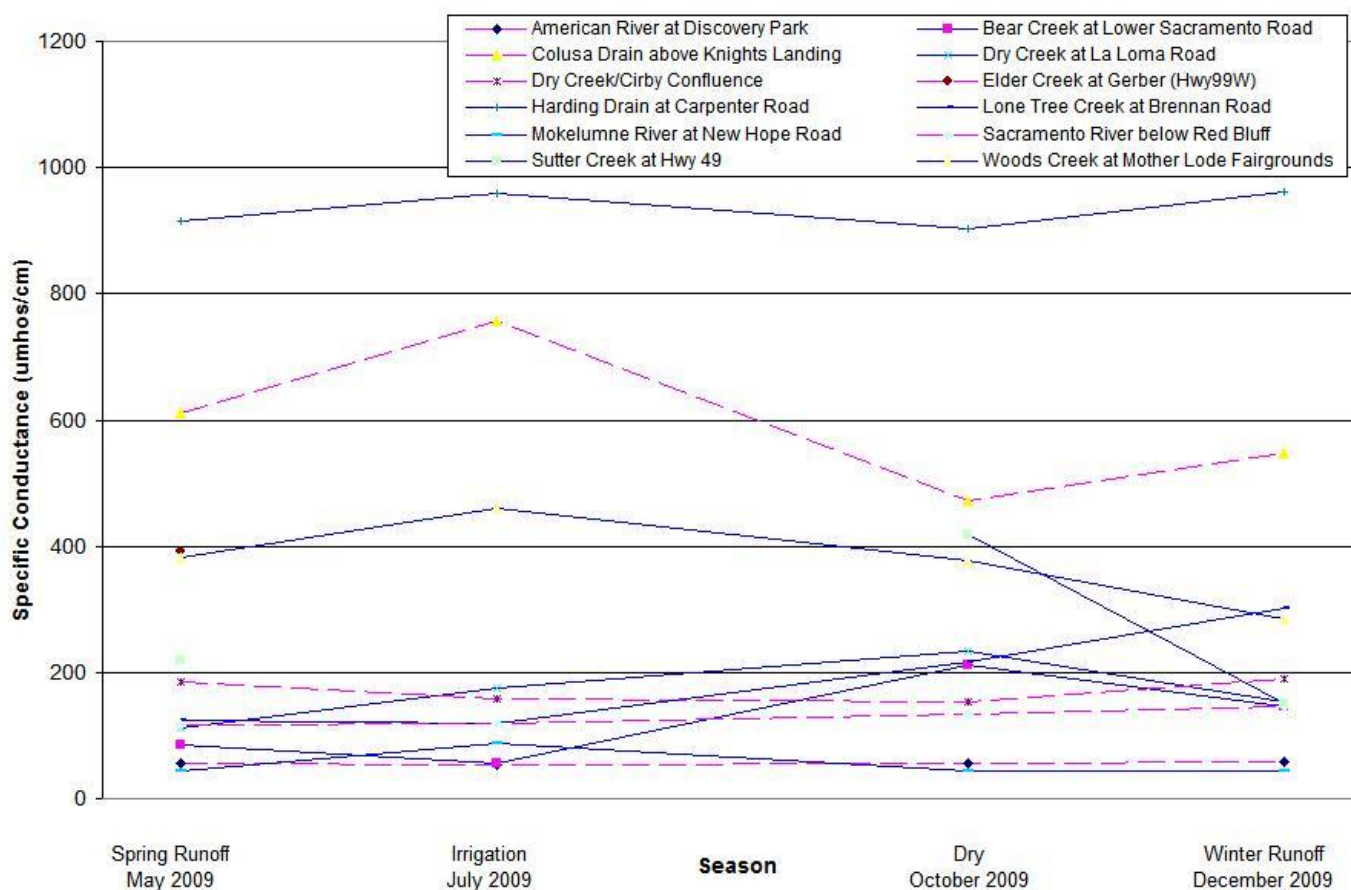
The overall pH range was 6.1 (measured at Sutter Creek) to 8.78 (measured at Mokelumne River at New Hope), both during the winter runoff sampling event. The overall pH range in the Sacramento River watershed sites was 6.9 to 8.43, with both results coming from American River at Discovery Park. All sites generally

experienced a slight drop in pH during the dry season, with the exception of Colusa Drain, where there was a slight increase from the pH during the irrigation season. The pH measured during the spring runoff, irrigation, and dry seasons were generally similar within each site, varying by less than half a pH unit, with exception of results measured at American River at Discovery Park, where results varied by as much as 1.53 pH units.

While results at Mokelumne River at New Hope Road and Bear Creek at Lower Sacramento displayed the similar dry season pH drop that was found at most Sacramento River watershed sites, sites in the San Joaquin River watershed displayed greater variation in ranges and seasonal patterns within each site than the Sacramento River watershed sites.

Specific Conductivity

Figure 6. Seasonal trends of water specific conductivity



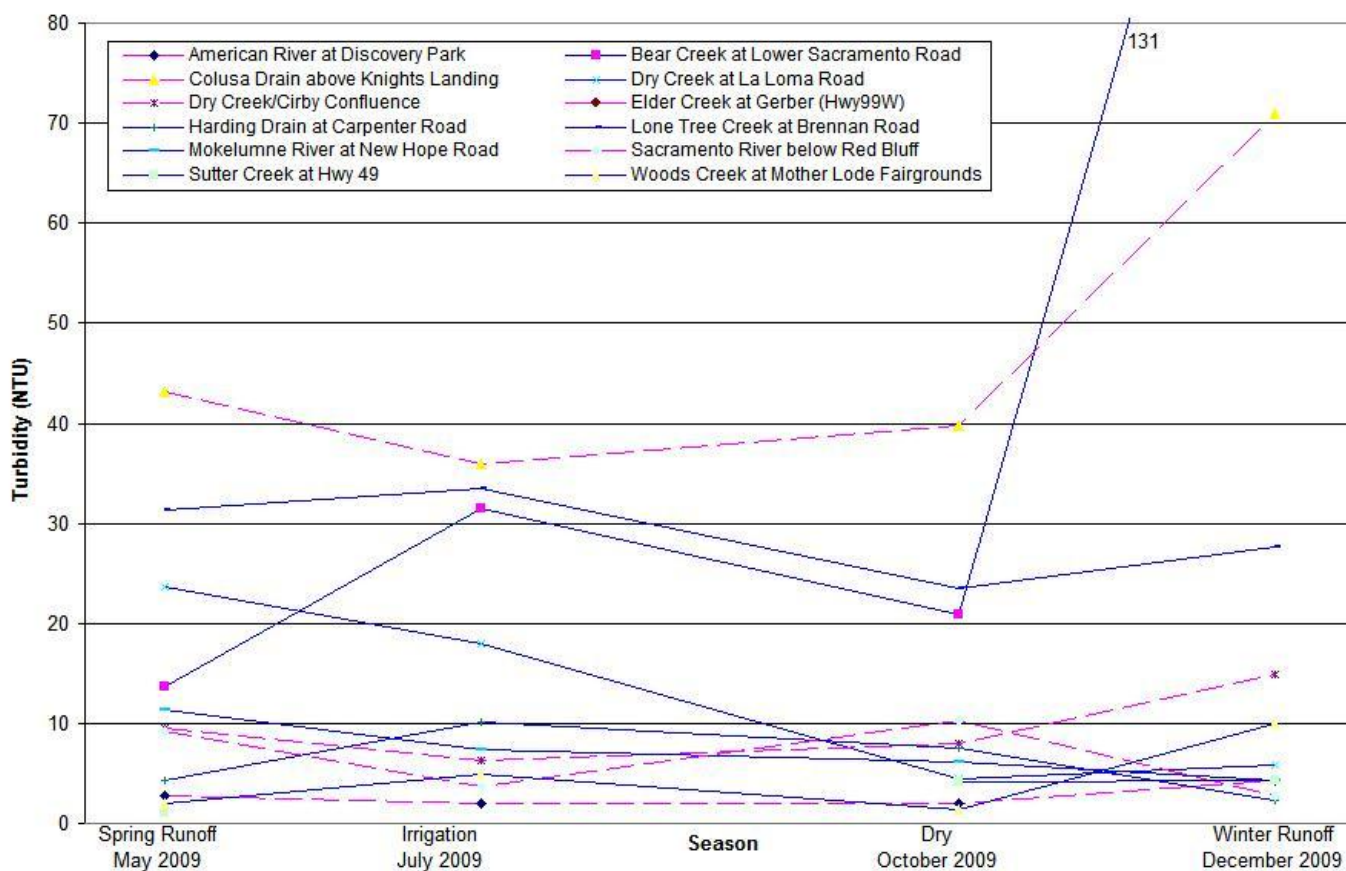
Seasonal patterns varied between most of the sites. However, the seasonal pattern for the two drain sites (Harding Drain and Colusa Drain) showed increase in concentration during the irrigation sampling event as compared to the spring runoff. Concentrations

then decreased during the dry season sampling event and then increased during the winter runoff. The magnitude of variation was more pronounced at the Colusa Drain site than Harding Drain.

Also noteworthy was the concentrations at Mokelumne River at New Hope. Concentrations were generally similar throughout the seasons, except during the July irrigation season sampling event, when results doubled from the typical 45 umhos/cm to 87 umhos/cm.

Turbidity

Figure 7. Seasonal trends of water turbidity



Six sites in both watersheds had increased turbidity during the irrigation season from concentrations during the spring runoff sampling event. Concentrations then decreased in half of the sites (Mokelumne River at New Hope, American River at Discovery Park, Dry Creek at La Loma), while concentrations increased at the other three sites (Sacramento River at Red Bluff, Colusa Drain above Knight's Landing, Dry Creek/Cirby Creek confluence).

At the four remaining sites where seasonal patterns could be evaluated (Bear Creek at Lower Sacramento River Road, Lone tree Creek at Brennan, Woods Creek at Mother Lode Fairgrounds, and Harding Drain at Carpenter Road), all of which were in the San Joaquin watershed, concentrations increased during the irrigation season from the spring runoff, but then decreased during the dry season in October.

During the winter runoff sampling event, results generally increased from the dry season results, with exception of results at Mokelumne River at New Hope, Sacramento River at Red Bluff, and Harding Drain, where results dropped.

E. coli

The seasonal concentrations of *E. coli* are listed in Table 8. *E. coli* were detected in all water samples that were analyzed from all sampling sites during each season. Average concentration of *E. coli* in the Sacramento River watershed locations was 120.6, 295.3, 263.6, and 184.9 MPN/100ml respectively for spring runoff, irrigation, dry and winter runoff seasons. For San Joaquin River watershed locations, data was >196.2, 543.3, 322.4, and >776.3 MPN/100 ml, respectively. The results for Lone Tree Creek during the spring and winter runoff seasons were above the reporting limit (>2419.6 MPN/100ml), so 2420 was used to calculate the mean and median for these seasons in the San Joaquin River watershed.

Using linear mixed effects regression (site set as a group effect due to repeated sampling, river and season set as independent variables, *E. coli* concentration as the dependent variable), there was not a significant difference between Sacramento and San Joaquin watershed ($P>0.5$) nor across season ($P>0.5$) for the concentration of *E. coli*.

Table 8. Seasonal concentration of *E. coli* (MPN/100ml)

Map ID	Station Number	Site Description	Spring runoff season (5/26/2009)	Irrigation season (7/20/2009)	Dry season* (10/19/2009)	Winter runoff season (12/14/2009)
Sacramento River Watersheds						
1	A0275890 (DWR SWCMP)	Sacramento River below Red Bluff	20.9	16	184.2	60.2
2	A0332000 (DWR SWCMP)	Elder Creek at Gerber	44.3	Dry	Dry	Dry
4	A0294710 (DWR SWCMP) 520CLSAKL (SWAMP ID)	Colusa Drain above Knights Landing	31.5	30.9	30.9	101.1
6	531PLA900 (SWAMP ID)	Dry Creek/Cirby Confluence	155.3	344.8	344.8	461.1

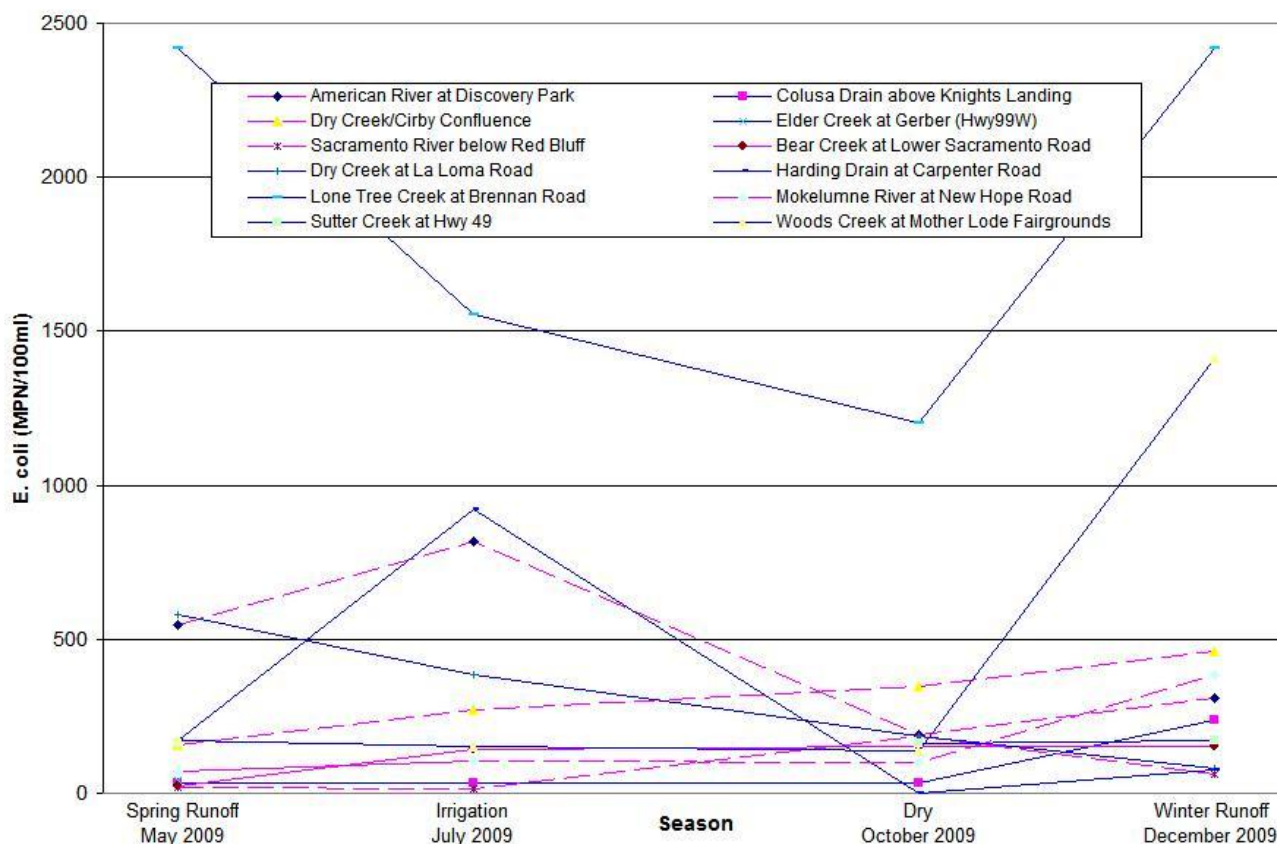
Map ID	Station Number	Site Description	Spring runoff season (5/26/2009)	Irrigation season (7/20/2009)	Dry season* (10/19/2009)	Winter runoff season (12/14/2009)
7	544SAC007 (SWAMP ID)	American River at Discovery Park	547.5	816.4	191.8	307.6
Arithmetic mean concentration			159.9	302.0	187.9	232.5
Median concentration			44.3	182.9	188.0	204.4
San Joaquin River Watersheds						
9	AMA002 (SWAMP ID)	Sutter Creek at Hwy 49	160.7	Dry	160.7	172.0
10	SAC002 (SWAMP ID)	Mokelumne River at New Hope Road	69.7	105	98.8	387.3
11	SJC515 (SWAMP ID)	Bear Creek at Lower Sacramento Road	22.8	142.1	150.0	151.5
12	TUO208 (SWAMP ID)	Woods Creek at Mother Lode Fairgrounds	172.2	151.6	137.6	1413.6
13	535XLTABR (ILRP ID)	Lone Tree Creek at Brennan Rd	>2419.6	1553.1	1203.3	2419.6
15	535XDCAWR (ILRP ID)	Dry Creek at La Loma Road	579.4	387.3	184.2	813.0
16	STC501 (SWAMP ID)	Harding Drain at Carpenter Road	172.3	920.8	Spilt sample	77.1
Arithmetic mean concentration			>513.8**	543.3	322.4	776.3
Median concentration			172.2	269.5	155.4	387.3

* A rain storm occurred during this sampling event, potentially influencing *E. coli* concentrations.

**The estimated mean and median concentrations used 2419.6 MPN/100 ml for the value of *E. coli* where results were above the reporting limit.

*** Results greater than the USEPA Designated Beach guideline (<235 MPN/100ml) are shaded yellow.

Figure 8. Seasonal concentration of *E. coli*



***E. coli* O157:H7**

The seasonal occurrence of *E. coli* O157:H7 is listed in Table 9. *E. coli* O157:H7 was only detected in two water samples during the entire project, one in May from Lone Tree Creek at Brennan Road, and the other one in July from the Sacramento River below Red Bluff. We were not able to detect a significant seasonal difference for the occurrence of this pathogen given the low number of positive samples (Fisher Exact Test, $P > 0.05$).

Table 9. Seasonal occurrence of *E. coli* O157:H7

Map ID	Station Number	Site Description	Spring runoff season (5/26/2009)	Irrigation season (7/20/2009)	Dry season* (10/19/2009)	Winter runoff season (12/14/2009)
Sacramento River Watersheds						
1	A0275890 (DWR SWCMP)	Sacramento River below Red Bluff	—	+	—	—

Map ID	Station Number	Site Description	Spring runoff season (5/26/2009)	Irrigation season (7/20/2009)	Dry season* (10/19/2009)	Winter runoff season (12/14/2009)
2	A0332000 (DWR SWCMP)	Elder Creek at Gerber	–	Dry	Dry	Dry
4	A0294710 (DWR SWCMP) 520CLSAKL (SWAMP ID)	Colusa Drain above Knights Landing	–	–	–	–
6	531PLA900 (SWAMP ID)	Dry Creek/Cirby Confluence	–	–	–	–
7	544SAC007 (SWAMP ID)	American River at Discovery Park	–	–	–	–
San Joaquin River Watersheds						
9	AMA002 (SWAMP ID)	Sutter Creek at Hwy 49	–	Dry	–	–
10	SAC002 (SWAMP ID)	Mokelumne River at New Hope Road	–	–	–	–
11	SJC515 (SWAMP ID)	Bear Creek at Lower Sacramento Road	–	–	–	–
12	TUO208 (SWAMP ID)	Woods Creek at Mother Lode Fairgrounds	–	–	–	–
13	535XLTABR (ILRP ID)	Lone Tree Creek at Brennan Rd	+	–	–	–
15	535XDCAWR (ILRP ID)	Dry Creek at La Loma Road	–	–	–	–
16	STC501 (SWAMP ID)	Harding Drain at Carpenter Road	–	–	–	–

+ and shaded yellow: positive; –: negative

* A rain storm occurred during this sampling event, potentially influencing the presence of *E. coli* O157:H7.

Salmonella

The seasonal occurrence and concentrations of *Salmonella* are listed in Table 10.

In the Sacramento River watershed, all sites had at least one season that tested positive for *Salmonella*, with an overall prevalence of 35% of water samples having detectable levels of this pathogen. Seasonal occurrence (MPN>0) at the 5 sites was 1/5, 0/5, 3/5, and 2/5 for spring runoff, irrigation, dry and winter runoff seasons, respectively. The Sacramento River at Red Bluff site was the only one where *Salmonella* was detected during more than one season (the first occurrence was during the dry season, with an increasing concentration in the winter runoff). The mean concentrations were 0.24, 0, 0.81, and 0.09 MPN/100ml for spring runoff, irrigation, dry and winter runoff seasons, respectively.

In the San Joaquin River watershed, results at the Sutter Creek at Hwy 49 (dry in irrigation season) and Dry Creek at La Loma Road sites were negative during all four seasons, but the remaining sites were positive for *Salmonella* during one or more seasons. The overall prevalence was 48% for water samples to have detectable levels of *Salmonella* in this watershed. Seasonal occurrence at the 7 sites was 2/7, 3/7, 4/7, and 4/7 for spring runoff, irrigation, dry and winter runoff seasons, respectively. The mean concentration was 0.12, 0.29, 0.10, and 0.13 MPN/100ml for the same 4 seasons, respectively.

Using linear mixed effects linear regression (site set as a group effect due to repeated sampling, river and season set as independent variables, *Salmonella* concentration as the dependent variable), there was no significant difference between Sacramento and San Joaquin watershed ($P=0.36$ given that season was in the model; $P=0.38$ when evaluated by itself) nor across season ($P=0.61$ given that river was in the model; $P=0.62$ when evaluated by itself). Converting *Salmonella* to presence (MPN>0) or absence (MPN=0) also did not find a significant association between the presence of this enteric bacteria and watershed location (Sacramento or San Joaquin) or season.

Table 10. Seasonal concentration of *Salmonella*

Map ID	Station Number	Site Description	Spring runoff season (5/26/2009)	Irrigation season (7/20/2009)	Dry season** (10/19/2009)	Winter runoff season (12/14/2009)
Sacramento River Watersheds						
1	A0275890 (DWR SWCMP)	Sacramento River below Red Bluff	0	0	0.09	0.18
2	A0332000 (DWR SWCMP)	Elder Creek at Gerber	1.2	Dry	Dry	Dry
4	A0294710 (DWR SWCMP) 520CLSAKL (SWAMP ID)	Colusa Drain above Knights Landing	0	0	0.46	0
6	531PLA900 (SWAMP ID)	Dry Creek/Cirby Confluence	0	0	2.67	0
7	544SAC007 (SWAMP ID)	American River at Discovery Park	0	0	0	0.18
Arithmetic mean concentrations			0.24	0	0.81	0.09
San Joaquin River Watersheds						
9	AMA002 (SWAMP ID)	Sutter Creek at Hwy 49	0	Dry	0	0
10	SAC002 (SWAMP ID)	Mokelumne River at New Hope Road	0	0	0.18	0.18
11	SJC515 (SWAMP ID)	Bear Creek at L. Sac. Road	0.46	0.46	0.18	0

Map ID	Station Number	Site Description	Spring runoff season (5/26/2009)	Irrigation season (7/20/2009)	Dry season** (10/19/2009)	Winter runoff season (12/14/2009)
12	TUO208 (SWAMP ID)	Woods Creek at Mother Lode	0	0	0.18	0.18
13	535XLTABR (ILRP ID)	Lone Tree Creek at Brennan Rd	0.37	0.09	0	0.09
15	535XDCAWR (ILRP ID)	Dry Creek at La Loma Road	0	0	0	0
16	STC501 (SWAMP ID)	Harding Drain at Carpenter Road	0	1.2	0.18	0.46
Arithmetic mean concentrations			0.12	0.29	0.10	0.13

* *Salmonella* concentrations were expressed as MPN/100ml

** A rain storm occurred during this sampling event, potentially influencing *Salmonella* concentrations.

*** Results that could be quantified are shaded yellow.

Bacteroidales

Table 11 shows the seasonal occurrence and concentrations of fecal contamination of monitoring sites in Sacramento River and San Joaquin River watersheds, as determined by analysis of *Bacteroidales*. According to *Bacteroidales* data fecal contamination was present in almost all samples (98% positives for universal *Bacteroidales* marker). Due to sites being dry or data being questionable, the following sites are not included in this discussion: Colusa Drain above Knight's Landing, Elder Creek at Gerber and Sutter Creek at Highway 49.

Universal *Bacteroidales* were detected in all sampling sites in all seasons which indicate the wide presence and prevalence of the universal fecal source bacteria. Overall for all sites, concentrations increased from the spring runoff to winter runoff, and results consistently increased at Sacramento River below Red Bluff. Concentrations generally increased moving from spring runoff to the irrigation season. Exceptions were Lone Tree Creek at Brennan Road, Mokelumne River at New Hope Road, and Dry Creek at the Cirby Creek confluence. Concentrations at these three sites then increased during the dry season sampling event, along with concentrations at Bear Creek at Lower Sacramento Road and American River at Discovery Park. Sites where concentrations decreased during the dry season after increasing from spring to the irrigation season were Harding Drain at Carpenter Road, Woods creek at Mother Lode Fairgrounds and Dry Creek at La Loma. During the winter runoff sampling event, results were split on whether concentrations increased or decreased from the dry season. Sites where concentrations increased were Mokelumne River at New Hope Road, Woods Creek at Mother Lode Fairgrounds, Dry Creek at La Loma, and Sacramento River below Red Bluff. At the remaining sites (Lone Tree Creek at Brennan Road, Dry Creek/Cirby Creek Confluence, Harding Drain at Carpenter Road, Bear Creek at Lower Sacramento Road, and American River at Discovery Park), concentrations decreased.

The most notable increases occurred during the irrigation season at Harding Drain at Carpenter Road and during the dry season at Lone Tree Creek at Brennan Road. Results for each of these samples were over one million gc/ml (1,000,000), while the next highest concentration throughout all the seasons was 107,000 gc/ml.

Cow *Bacteroidales* were mostly found in the San Joaquin River watershed sites. The only time they were found in the Sacramento River watershed sites was during the dry season at the Dry Creek/Cirby Creek confluence. Lone Tree Creek at Brennan Road was the only site where cow *Bacteroidales* were found during all four sampling events. Concentrations decreased from spring thru the dry season sampling events. During the winter sampling event, there was a slight bump in the concentration.

Human *Bacteroidales* were mostly found at Woods Creek at Brennan Road. The concentration generally increased through the study, although there were none detected during the dry season sampling event.

Dog *Bacteroidales* were only found during the spring runoff sampling event; and then only at a concentration at least a thousand times lower than any of the other *Bacteroidale* results.

Table 11. Seasonal Bacteroidale occurrence and concentrations

Map ID	Station Number	Site Description	Spring runoff (5/26/2009)				Irrigation (7/20/2009)				Dry* (10/19/2009)				Winter runoff (12/14/2009)			
Sacramento River Watersheds																		
1	A0275890 (DWR SWCMP)	Sacramento River below Red Bluff	U** 0.7				U 3.0				U 10				U 16			
2	A0332000 (DWR SWCMP)	Elder Creek at Gerber	U 10				Dry				Dry				Dry			
4	A0294710 (DWR SWCMP) 520CLSAKL (SWAMP)	Colusa Drain above Knights Landing	U 1.0				U 1.2				Not detected				U 68			
6	531PLA900 (SWAMP)	Dry Creek/Cirby Confluence	U 19			H 0.062	U 4.2				U 94	C 1.3			U 55			
7	544SAC007 (SWAMP)	American River at Discovery Park	U 1.5				U 2.3				U 7.6				U 7.2			
San Joaquin River Watersheds																		
9	AMA002 (SWAMP)	Sutter Creek at Hwy 49	U 5.3	C 0.08			Dry				U 30				U 15			
10	SAC002 (SWAMP)	Mokelumne River at New Hope Road	U 3.5				U 1.1				U 8.1				U 22			
11	SJC515 (SWAMP)	Bear Creek at Lower	U 9.5				U 18				U 39				U 24			

Map ID	Station Number	Site Description	Spring runoff (5/26/2009)				Irrigation (7/20/2009)				Dry* (10/19/2009)				Winter runoff (12/14/2009)			
		Sacramento Road																
12	TUO208 (SWAMP)	Woods Creek at Mother Lode Fairgrounds	U 4.8			H 0.9	U 6.0	C 2.1		H 1.5	U 0.7				U 81	C 0.22		H 5.0
13	535XLTABR (ILRP ID)	Lone Tree Creek at Brennan Rd	U 51	C 16	D 16		U 36	C 8.0			U 1,078	C 1.1			U 161	C 1.8		
15	535XDCAWR (ILRP ID)	Dry Creek at La Loma Road	U 8.8	C 0.22			U 48	C 0.16			U 11				U 19			
16	STC501 (SWAMP)	Harding Drain at Carpenter Road	U 6.8				U 1,157	C 2.8			U 107	C 0.41			U 17			

* A rain storm occurred during this sampling event, potentially influencing the identified sources of fecal contamination.

** U = Universal *Bacteroidales*; C = Cow *Bacteroidales*; D = Dog *Bacteroidales*; H = Human *Bacteroidales*. Concentrations listed in thousands of gc/ml for universal, cow, and dog *Bacteroidales*.

Concentration for dog *Bacteroidales* listed as original result

*** Results that could be quantified are shaded yellow.

Objective 2: Determine whether *E. coli* O157:H7 and *Salmonella* are present at any time at the sites being evaluated

Although there were relatively high levels of *E. coli* at the various sampling sites, only 2 samples had detectable levels of *E. coli* O157:H7.

The first site was Lone Tree Creek at Brennan Road during the spring runoff. This water sample also had one of the highest level of indicator *E. coli* during the project (>2419.6 MPN/100 ml), tested positive for *Salmonella*, and also tested positive for cow and dog fecal sources during this sampling event.

The other site that tested positive for *E. coli* O157:H7 was Sacramento River below Red Bluff during July 2009. Interestingly, and quite opposite the trends of Lone Tree Creek, this water sample had very low levels of indicator *E. coli* (16 MPN/100 ml), tested negative for *Salmonella*, and did not have a species-specific fecal source detected by the *Bacteroidales* method. However, as noted in the seasonal trends discussion, during this sampling event, while water temperature followed the expected seasonal pattern, DO results were unexpectedly high. In future monitoring projects it might be helpful to increase the sampling volume to 1, 5 or 10 liters in order to increase the sensitivity of the assay. Nonetheless, given that we processed 500 ml and only detected 2 positive samples suggests that this pathogenic strain of *E. coli* is not common at concentrations above 5 to 10 cfu/100 ml given the large number of negative results.

In contrast to *E. coli* O157:H7, *Salmonella* was detected in about a third (35%) of the samples from the Sacramento watershed sites and about half (48%) of the San Joaquin sampling sites, with concentrations ranging from 0.09 to 2.7 MPN/100 ml.

Objective 3: Evaluate potential sources of fecal contamination and at a minimum group potential sources to human, cattle, or other animals

Almost all water samples (98%) had the universal fecal source *Bacteroidales* marker. In contrast, only about 6% of the water samples from the Sacramento monitoring sites had detectable human fecal sources, and, about 6% of water samples had cow fecal sources.

This low level of definitive fecal source tracking does not provide a concrete recommendation as to key fecal sources at these monitoring sites. A higher frequency of sampling might improve this; alternatively, these results could indicate that the major fecal sources for these sites are not human, cow, nor dog.

With respect to the San Joaquin monitoring sites, about 11% and 37% of water samples had human and cow fecal sources, respectively. The relatively frequent occurrence of human fecal sources (3/4 positive) at Woods Creek at Mother Lode Fairgrounds (Community Development) and cow fecal sources (4/4 positive) at Lone Tree Creek at Brennan Rd (Irrigated Agriculture, Community Development, Semi-agricultural and Incidental to Agriculture) indicate that human and bovine activity upstream of these monitoring sites may be contributing a portion of the microbial species (*E. coli* O157:H7, *Salmonella*) and indicator *E. coli* isolated from these locations.

Objective 4: Document the presence of source identifier DNA in viable vs. non-viable *Bacteroidales* cells in relation to the presence of *E. coli*, *E. coli* O157:H7, and *Salmonella*

The assay for distinguishing viable from non-viable *Bacteroidales* cells generated questionable results, as discussed in the QA section of this report, hence, this discussion only focuses on the results regarding identification of fecal sources from various vertebrate species (human, cow, dog, other).

Concentrations of *E. coli* were significantly higher for the group of water samples with a cow fecal source or a human fecal source, but this relationship did not occur for *Salmonella*. It is possible that the environmental or land-use conditions that lead to higher levels of indicator *E. coli* may also lead to elevated levels of fecal contamination from these two sources. Interestingly, the land use designation for each site was not associated with the likelihood of finding human or cow fecal sources, hence, it is likely some other factor (e.g., site-specific, climate) may be causing the fecal contamination.

The two occurrences of *E. coli* O157:H7 did not coincide with a specific vertebrate source or land use designation. The positive sample from Sacramento River below Red Bluff (integrator/recreation site) occurred during the summer irrigation season with the water sample having only universal *Bacteroidales* as the fecal source (i.e., non-human, non-cow, non-dog source); Lone Tree Creek at Brennan Road (irrigated ag/community develop/semi-agricultural and incidental to agriculture) positive sample occurred during the spring runoff from a water sample having both dog and cow fecal signatures.

Using mixed effects Poisson regression (site set as the group random effect due to repeated sampling, cow or human fecal source as the independent variable, and either *E. coli* or *Salmonella* as the dependent variable), water samples with a cow fecal source had significantly higher concentrations of *E. coli* ($P<0.001$) but not *Salmonella* ($P=0.12$). Water samples with a human fecal source had significantly higher concentrations of *E. coli* ($P<0.001$) but not *Salmonella* ($P=0.51$) (Table 12).

Table 12. Association between water with cow or human fecal sources and *E. coli*, and *Salmonella*

Fecal source	<i>E. coli</i> concentration*	<i>Salmonella</i> concentration*
Cow		
<i>present</i>	1057	0.42
<i>absent</i>	193	0.13
Human		
<i>present</i>	479	0.045
<i>absent</i>	407	0.22

* MPN/100 ml

Objective 5: Compare reported concentrations to appropriate water quality objectives and guidelines including the Central Valley Regional Board Basin Plan (Basin Plan, 2007) and the USEPA Bacterial Water Quality Standards for Recreational Waters guidelines (USEPA Standards, 2003)

Parameters for indicators of Beneficial Use protection were drawn from the Central Valley Water Board Basin Plan, Calfed Guidelines, USEPA Criteria, State Board Objectives, and recommendations from the Food and Agriculture Organization of the United Nations and Water Quality for Agriculture. Table 13 summarizes the parameter used to determine whether beneficial uses were protected for each of the constituents measured.

Table 13. Water Quality Objectives, Guidelines, and Recommendations used to evaluate data

Parameter	Recreation	Drinking Water	Aquatic Life	Agriculture
Water temperature			Calfed Guideline (20-C) CVRWQCB Basin Plan, Sacramento River from Shasta Dam to I Street Bridge (13-C at Sac R. Below Red Bluff, Elder Creek, Dry/Cirby Creeks) Time Period for both: Apr 1 – Jun 30 & Sep 1 – Nov 30	
Dissolved Oxygen			CVRWQCB Basin Plan Waters designated	

Parameter	Recreation	Drinking Water	Aquatic Life	Agriculture
			WARM (5.0 mg/L) Waters designated COLD and SPWN (7.0 mg/L) At Sac R. Below Red Bluff and Elder Creek, Time Period for Sac R & Elder Creek only: 1 Jun to 31 Aug (9.0 mg/L)	
pH	USEPA National Ambient water Quality Criteria (5-9)	USEPA Secondary MCL (6.5-8.5)	CVRWQCB Basin Plan (6.5 – 8.5)	Food & Ag Org. of United Nations (6.5-8.4)
Conductivity		CVRWQCB Basin Plan Maximum Contaminant Level Ranges Recommend – 900 umhos/cm Upper – 1600 umhos/cm Short term – 2200 umhos/cm		Water Quality for Agriculture (Ayers & Westcot) 700
Turbidity			State Board Objective for Human Health Protection, fish consumption only Instantaneous Maximum 225	
<i>E. coli</i>	CVRWQCB Basin Plan Fecal Coliform (400 MPN/100ml) EPA Rec Guideline Desig. Beach (235 MPN/100ml) Moderate Use (298 MPN/100ml) Light Use (409 MPN/100ml) Infrequent Use (575 MPN/100ml)			
<i>E. coli</i> O157:H7				
Bacteroidales				
<i>Salmonella</i>				

The Central Valley Water Board Basin Plan identifies beneficial uses to be applied to surface water bodies. The beneficial uses for the sites selected for this study are summarized in table 14. Where water bodies are not specifically identified in the Basin Plan, designated beneficial uses in the first downstream water body are generally applied, and indicated as “Tributary Rule”.

This means of specifying beneficial uses applies only to “streams” and not to “constructed agricultural drains.” The beneficial uses for constructed agricultural drains are used as guidelines unless designated by a plan or policy. While the beneficial uses listed for the Colusa Drain are specified in the Basin Plan, they are not for Harding Drain. The beneficial uses listed for Harding Drain are consistent with the report titled Water Quality of the San Joaquin River and Major Drainage Basins, October 2000-September 2005.

Table 14 Applicable beneficial uses for the Central Valley Bacteria Source Identification Screening Study

Basin Plan Table II-1 Column 1	Site Description	MUN	AGR	RECREATION			FRESHWATER HABITAT		MIGRATION		SPWN		Designated (D) or Tributary Rule (T)
		Municipal and Domestic Supply	Irrigation	REC-1		REC-2	WARM	COLD	MIGRATION		WARM	COLD	
				Contact	Canoeing and Rafting	Other Non Contact	Warm	Cold	Warm	Cold	Warm	Cold	
13	Sacramento River Below Red Bluff	E	E	E	E	E	E	E	E	E	E	E	D
13	Elder Creek at Gerber	E	E	E	E	E	E	E	E	E	E	E	T
29	Colusa Basin Drain above Knights Landing		E	E	E		E	P	E		E		D
30	Dry Creek/Cirby Confluence	E	E	E	E	E	E	E	E	E	E	E	T
51	American River at Discovery Park	E	E	E	E	E	E	E	E	E	E	E	D
63	Sutter Creek at Hwy 49		E	E	E	E	E	E	E	E	E	E	T
63	Mokelumne River at New Hope Road		E	E	E	E	E	E	E	E	E	E	D
C	Bear Creek at Lower Sacramento Road	E	E	E		E	E	E	E	E	E		T
84	Woods Creek at Mother Lode Fairgrounds	E	E	E	E	E	E	E					T

Basin Plan Table II-1 Column 1	Site Description	MUN	AGR	RECREATION			FRESHWATER HABITAT		MIGRATION		SPWN		Designated (D) or Tributary Rule (T)
		Municipal and Domestic Supply	Irrigation	REC-1		REC-2	WARM	COLD	MIGRATION				
				Contact	Canoeing and Rafting	Other Non Contact	Warm	Cold	Warm	Cold	Warm	Cold	
C	Lone Tree Creek at Brennan Road	E	E	E		E	E	E	E	E	E		T
90	Dry Creek at La Loma Road	P	E	E	E	E	E	E		E	E	E	T
83	Harding Drain at Carpenter Road	E	E	E	E	E	E	E	E	E	E	E	WDR Order R5- 2010- 0002

Drinking Water (pH, Specific Conductivity, *E. coli*)

Indicators used to evaluate a potential impact to drinking water (sources of municipal and domestic supply) included pH (6.5 - 8.5), salt measured as specific conductivity (900 umhos/cm), and *E. coli*. For all the indicators except *E. coli*, there are specific numeric objectives or goals for drinking water that can be evaluated against (Table 13). There are no specific numeric criteria for *E. coli* related to consumption but the presence of *E. coli* would indicate that the water would need to be treated prior to consumption.

While Sutter Creek at Highway 49 and Mokelumne River at New Hope Road are exempt per table II-1 in the Basin Plan, the State Board drinking water policy (State Board Resolution No. 88-63 and Basin Plan, 2006) overrides this and makes them drinking water supplies. Drinking water is a potential beneficial use at Dry Creek at La Loma Road.

All results for pH and SC were within the recommendations with exceptions of elevated pH at Mokelumne at New Hope Road during the irrigation and winter runoff seasons, and low pH at Sutter Creek during the dry and winter runoff seasons. Specific conductance was only elevated at the Harding Drain site, but was relatively consistent throughout the monitoring season, ranging from 902 - 961 umhos/cm. Additionally, *E. coli* was present in all samples at all sites.

Irrigation Water Supply (pH, Specific Conductivity)

All sites were included in evaluation with irrigation being an existing beneficial use.

For specific conductivity, the Basin Plan objectives only apply to running averages. Therefore, the Water Quality for Agriculture goal of 700 umhos/cm was used to evaluate data. Also, the Food and Agriculture Organization of the United Nations pH goal of 6.5 – 8.4 was used.

There were only four sites where results were outside of these parameters:

- Colusa Drain above Knight's Landing (SC of 757 umhos/cm during the July sampling event)
- Mokelumne River at New Hope Road (pH at 8.62 during the July sampling event and 8.78 during the December sampling event)
- Sutter Creek at Highway 49 (pH at 6.1 during the October and December sampling events)
- Harding Drain at Carpenter Road (SC above 900 umhos/cm during all sampling events).

Aquatic Life (Water temperature, Dissolved Oxygen, pH, Turbidity)

Results were compared to the following objectives and guidelines to evaluate the aquatic life beneficial use:

- Water temperature below 20-C at all sites except Sacramento River Below Red Bluff, Elder Creek, and Dry/Cirby Creek Confluence. For these three sites, the Basin Plan required temperature to be below 13-C. The time frame for these parameters was April 1 thru June 30 and September 1 thru November 30 (May and October sampling events)
- Dissolved Oxygen was compared to objectives specified in the Basin Plan based on the beneficial use designations of WARM (5.0 mg/L), COLD (7.0 mg/L), and SPWN (7.0 ml/L). Additionally, Sacramento River Below Red Bluff and Elder Creek at Gerber had an additional objective of 9.0 mg/L 1 June thru 31 August.
- The Basin Plan objective for pH was 6.5 – 8.5.
- The instantaneous maximum State Board turbidity objective was 225 NTU.

Warm and cold freshwater habitat, migration, and spawning are existing beneficial uses for Sacramento River below Red Bluff, Elder Creek at Gerber, Dry Creek/Cirby Creek confluence, American River at Discovery Park, Sutter Creek at Highway 49, and Mokelumne River at New Hope Road. Bear Creek and Lone Tree Creek have aquatic life beneficial use for all sub categories except cold spawning. Dry Creek at La Loma only excludes warm migration aquatic life subcategory. Harding Drain excluded cold freshwater and cold spawning. Colusa Drain excluded cold migration and cold spawning, and cold freshwater habitat is a potential beneficial use. At Woods Creek at Mother Lode Fairgrounds, only warm and cold freshwater habitat is existing beneficial uses.

While water temperature at the Sacramento River below Red Bluff and the Dry/Cirby Creek confluence met the Calfed guideline, these temperatures were above the Basin Plan objective. Water temperature at Elder Creek was also above the Basin Plan objective, as well as the Calfed guideline. Water temperature was also above the Calfed guideline (only during the May sampling event) at Bear Creek at Lower Sacramento Road, Lone tree Creek at Brennan Road, Dry Creek at La Loma Road, and Harding Drain at Carpenter Road.

The dissolved oxygen WARM objectives of 5.0 mg/l were not met at four of the twelve sampling sites at the times indicated below:

- Colusa Drain above Knight's Landing during the May and July sampling events,
- Sutter Creek at Highway 49 during the October sampling event,
- Bear Creek at Lower Sacramento Road during the May and October sampling events,
- Dry Creek at La Loma during the December sampling event.

Additional sites and results did not meet the cold and spawning objective of 7.0 mg/l:

- Colusa Drain above Knight's Landing during the October sampling event
- Bear Creek at Lower Sacramento Road during the July sampling event
- Lone Tree Creek at Brennan Road during the October and December sampling events
- Dry Creek at La Loma Road during the October sampling event

All results met the turbidity objective.

Recreation (*E. coli*, *E. coli* O157:H7, *Salmonella*, *Bacteroidales*)

E. coli is widely used as an indicator to determine the likelihood of pathogens in water column. The current Basin Plan WQO focuses on fecal coliform concentrations (<200 MPN/100ml for a 5-day geometric mean or <400 MPN for a single sample). Analyses for this study utilized *E. coli*, a subset of fecal coliform. Use of *E. coli* allowed both a conservative evaluation against the Basin Plan WQO as well as a comparison to USEPA guidelines for various levels of recreational contact, as detailed in Table 13. The Central Valley Water Board Basin Plan identifies contact recreation as a beneficial use throughout the Region.

About 12% and 35% of water samples from the Sacramento River watershed exceeded the Basin Plan objectives and EPA designated beach standards, respectively. In addition, about 18% and 24% of water samples from the San Joaquin River watershed exceeded the Basin Plan objectives and EPA designated beach standards, respectively.

Table 15. Comparison of concentrations of *E. coli* to water quality objectives and guidelines

Comparison to State Board Basin Plan and EPA guideline		Spring runoff season (5/26/2009)	Irrigation season (7/20/2009)	Dry Season* (10/19/2009)	Winter runoff season (12/14/2009)
Sacramento River Watershed					
Basin Plan objective (fecal coliform not to exceed 400 MPN/100ml in >10% of samples)**	% of sites exceed objective	0% (0/5)	25% (1/4)	25% (1/4)	0% (0/4)
EPA guideline (<i>E. coli</i> not to exceed 235MPN/100ml)	% of sites exceed guideline	20% (1/5)	50% (2/4)	50% (2/4)	25% (1/4)
San Joaquin River Watershed					
Basin Plan objective (fecal coliform not to exceed 400 MPN/100ml in >10% of samples)**	% of sites exceed objective	28.6% (2/7)	33% (2/6)	16.7% (1/6)	43% (3/7)
EPA guideline (<i>E. coli</i> not to exceed 235MPN/100ml)	% of sites exceed guideline	28.6% (2/7)	50% (3/6)	16.7% (1/6)	57.1% (4/7)

* A rain storm occurred during this sampling event, potentially influencing *E. coli* concentrations.

**This project sampled each site only 4 times over 12 months which does not match the 30-day time frame of the Basin Plan for fecal coliform monitoring. Sites with one or more samples (25% or more) having >400 *E. coli* MPN/100 ml were classified as exceeding the water quality objective.

Using mixed effects logistic regression to identify one more factors associated with exceeding the *E. coli* 235 standard, we found that the presence of cow fecal source as determined by the *Bacteroidales* assay was significantly associated with exceeding the 235 MPN/100 ml standard for *E. coli* ($P=0.009$). Specifically, the odds of exceeding this EPA beach standard was about 29 times larger when cow fecal sources were detected (OR=28.8, 95% CI 2.34-355) compared to water samples where no fecal sources were detected. In contrast, watershed location (Sacramento versus San Joaquin), season, presence of human fecal sources, or any of the land use designations at each site (irrigated agriculture, CAFO, etc.) were not significantly associated ($P>0.05$) with exceeding the EPA beach standard.

Using mixed effects logistic regression to identify one more factors associated with exceeding the fecal coliform 400 standard (using *E. coli* as a proxy), we found that the presence of cow fecal source as determined by the *Bacteroidales* assay was significantly associated with exceeding this standard ($P<0.001$). Specifically, the odds of exceeding this Basin Plan objective was about 40 times larger when cow fecal sources were detected (OR=40, 95% CI 5.7-282) compared to water samples where no cow

fecal sources were detected. In contrast, watershed location, season, presence of human fecal sources, or any of the land use designations at each site (irrigated agriculture, CAFO, etc.) were not significantly associated ($P>0.05$) with exceeding the Basin Plan objective.

The reason such a finding is not definitive is that there are many vertebrate and environmental sources of *E. coli* that are not detected at the species level by the *Bacteroidales* method, and if these sources co-locate with cattle sources (e.g., horses, sheep, other domestic or wild animals) then such a finding is possible. Nonetheless, if one goal of such technology is to identify the geographical location causing the water quality impairment, then finding cattle *Bacteroidales* should help narrow the initial focus of which land owners may be causing the largest *E. coli* loadings into these sites. For example, the consistent cow *Bacteroidales* (4/4 positive) at Lone Tree Creek at Brennan Rd (Irrigated Agriculture, Community Development, Semi-agricultural and incidental to agriculture) is strongly supportive that bovine activity upstream of this monitoring sites may be contributing a portion of the microbial species (*E. coli* O157:H7, *Salmonella*) and indicator *E. coli* isolated from this location.

Although the prevalence and concentration of *Salmonella* were slightly higher for water samples that exceeded either the EPA designated swimming beach standard or the Basin Plan objective compared to water samples that did not exceed these standards (Table 16), these differences were not statistically significant. It is possible that a larger sample size would have generated sufficient statistical power to detect a significant difference.

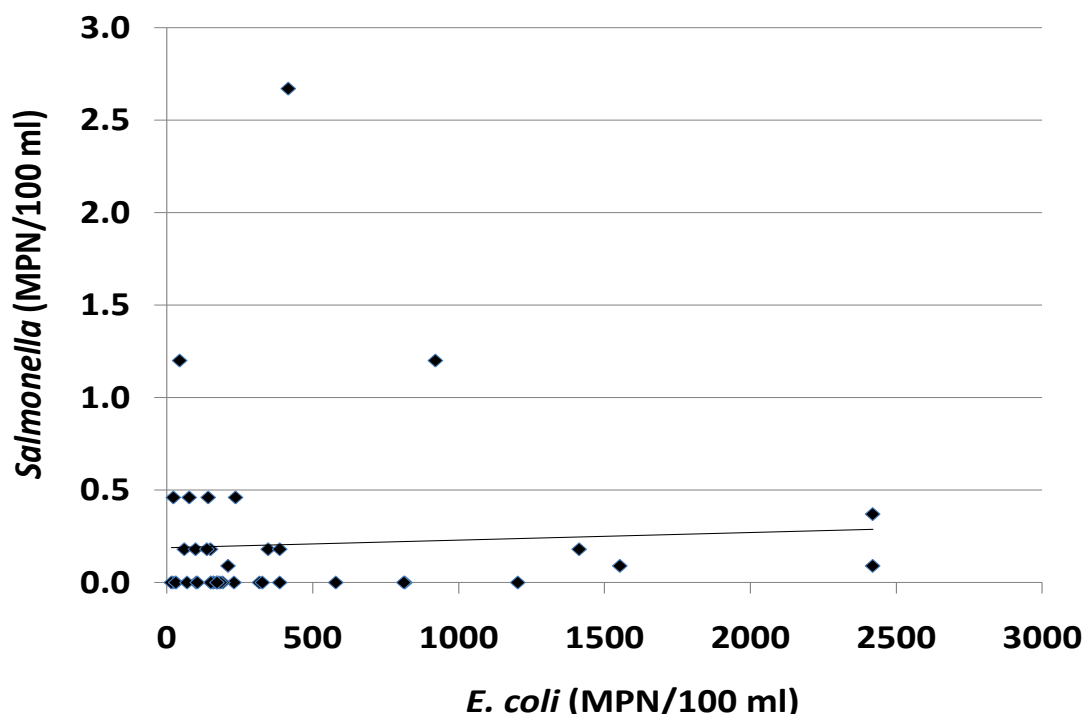
Table 16. Comparison of *Salmonella* in water samples that exceeded or did not exceed the *E. coli* standards

<i>E. coli</i> standard*	<i>Salmonella</i> prevalence**	<i>Salmonella</i> concentration**
EPA beach		
>235 MPN	56%	0.339
<235 MPN	33%	0.126
Basin Plan		
>400 MPN	60%	0.46
<400 MPN	36%	0.128

* This project sampled each site only 4 times over 12 months which does not match the 30-day time frame of the Basin Plan for fecal coliform monitoring. Sites with one or more samples (25% or more) having >400 *E. coli* MPN/100 ml were classified as exceeding the water quality objective.

** Prevalence of *Salmonella* was calculated as number of samples having MPN>0 divided by all samples. Concentration of *Salmonella* was the arithmetic mean (MPN/100 ml) of all samples.

Figure 9. The lack of an association between *E. coli* and *Salmonella*



Using mixed effects Poisson regression (site set as a group effect due to repeated sampling, *E. coli* concentration as the independent variable, *Salmonella* concentration as the dependent variable), the concentration of *E. coli* was not associated with the concentration of *Salmonella* ($P=0.73$). The scatter plot of *E. coli* versus *Salmonella* is shown in Figure 1 below. We do not know the human virulence of the *Salmonella* in these water samples, but using a human dose-response curve for *Salmonella* estimated by the World Health Organization estimates that ingestion of 100 ml of water containing 3 MPN/100 ml would result in a risk of human infection of about 0.02% (2 in 10,000). The importance of the observation that there is a lack of association between *E. coli* and *Salmonella* concentrations is that *E. coli* cannot be used as a surrogate to evaluate risk to human health of *Salmonella*.

Aim 6: Make recommendations for future bacteria source identification studies

1. The small sample size per site in this project resulted in low statistical power to detect seasonal and geographical differences in *E. coli*, *E. coli* O157:H7 and *Salmonella* for these two watersheds. This was due in part from the high cost per sample of conducting the *Bacteroidales* assay. Depending on the goals of the project, it may be prudent to hold the DNA (assuming it is sufficiently stable over 12 months) until the bacterial monitoring is completed. One can then determine which sites during which seasons exhibit high microbial levels and apply this technology to just those problematic samples for specific sites at specific seasons.

2. Based on the findings of the occurrence of *E. coli* O157:H7 and *Salmonella*, we would recommend conducting future studies to address more intensive spatial and temporal sampling at certain sites such as sampling the at Sacramento River below Red Bluff and Lone Tree Creek at Brennan Road twice a month for at least 12 months. We would also consider including additional molecular targets, such as *E. coli* stx1, stx 2, and rfbE in future studies.

3. We will improve the current methodology for *Bacteroidales* and bacterial viability analyses. We would establish a reverse transcription PCR (RT-PCR) technique for bacterial viability assay. The RT-PCR amplifies mRNA which is degraded rapidly upon cell death (half-life from seconds to some minutes). Therefore, the presence of mRNA is believed to be a valid and sensitive indicator of viable cells. Given that DNA sequence polymorphisms and pulsed-field gel electrophoresis (PFGE) are among the methods for Microbial Source Tracking (MST) (Foley et al., 2009), we can develop a combined methods of DNA sequencing and PFGE for MST in water samples. Source of bacteria will be determined by online comparisons of sequences of DNA amplified from indicator *E. coli* to the published sequences from humans and animals combined with comparisons of PFGE patterns with representative *E. coli* strains from different host species.

4. Because of the limit of sample numbers and sampling frequency as well as the challenges in technology, we can hardly draw conclusions regarding to the source of fecal contamination and correlations between fecal sources and bacterial concentrations in water. We would recommend that the volume of water used for detecting *Salmonella* and *E. coli* O157:H7 be increased to at least 10 liter, and possibly consider 50 liters. We evaluated the utility of an in-field tangential ultrafiltration method to concentrate these larger volumes of water. We found this method is feasible for rapid concentrating of 10-50 liter of well water and we are using this method in a current USDA project. An example of this method is described in Applied and Environmental Microbiology 2005, 71(11): 6878–6884.

5. There are no water quality standards to measure the hazard to human health for bacteria other than *E. coli*, Enterococci, and fecal coliform. Epidemiology studies would provide valuable information on concentration load limits for bacteria, including *Salmonella* and *E. coli* O157. We would consider including additional molecular targets, such as *E. coli* stx1, stx 2, and rfbE in future studies to better understand the virulence and toxins produced by *E. coli* O157. We are also interested in investigating the associations of land use to the occurrence of pathogenic bacteria in watersheds. Example issues to address include species and size of farm, practice of antibiotic uses, practice of manure management, buffers (if any) properties, and proximity to watersheds.

8.0 CONCLUSION

This study allowed for a preliminary investigation of including Microbial Source Tracking methodology in water quality monitoring conducted by the Central Valley Water Board and relating this monitoring to previous monitoring efforts.

Although there was substantial variability in the concentration of *E. coli* across the four different sampling seasons in this project for each set of sites with the Sacramento and San Joaquin watersheds, this variability was not significantly different when subjected to statistical analysis. The most likely explanation is the small sample size per site and the typical high variability for these indicator bacteria between sites, which makes finding statistical differences difficult. Concentrations of *E. coli* often varied in excess of 5- to 10-fold between sites for the same season for each watershed. A rain event did occur during the sampling for the Dry season, potentially altering the *E. coli* levels. Despite this lack of a significant difference across season, many sites exceeded water quality standards based on either the Basin Plan objectives or EPA's standard for beach swimming, as discussed below.

The persistent and high concentrations of *E. coli* at site 13 (Lone Tree Creek on Brennan Road) are of concern and warrant a more in-depth investigation as to the cause of the water quality impairment. Moreover, this site was one of the few sites that tested positive for *E. coli* O157:H7, had persistent levels of *Salmonella*, and also had persistent cow and one dog fecal source as indicated by the *Bacteroidales* method. Although finding a specific fecal source in a watershed does not prove which animal species is causing the water quality impairment, it does suggest that cattle may be a contributor to the elevated bacterial counts and that an upstream livestock operation may be a key contributor of indicator bacteria to this monitoring site.

9.0 REFERENCES

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Figure 10. Family tree: Kingdom Bacteria

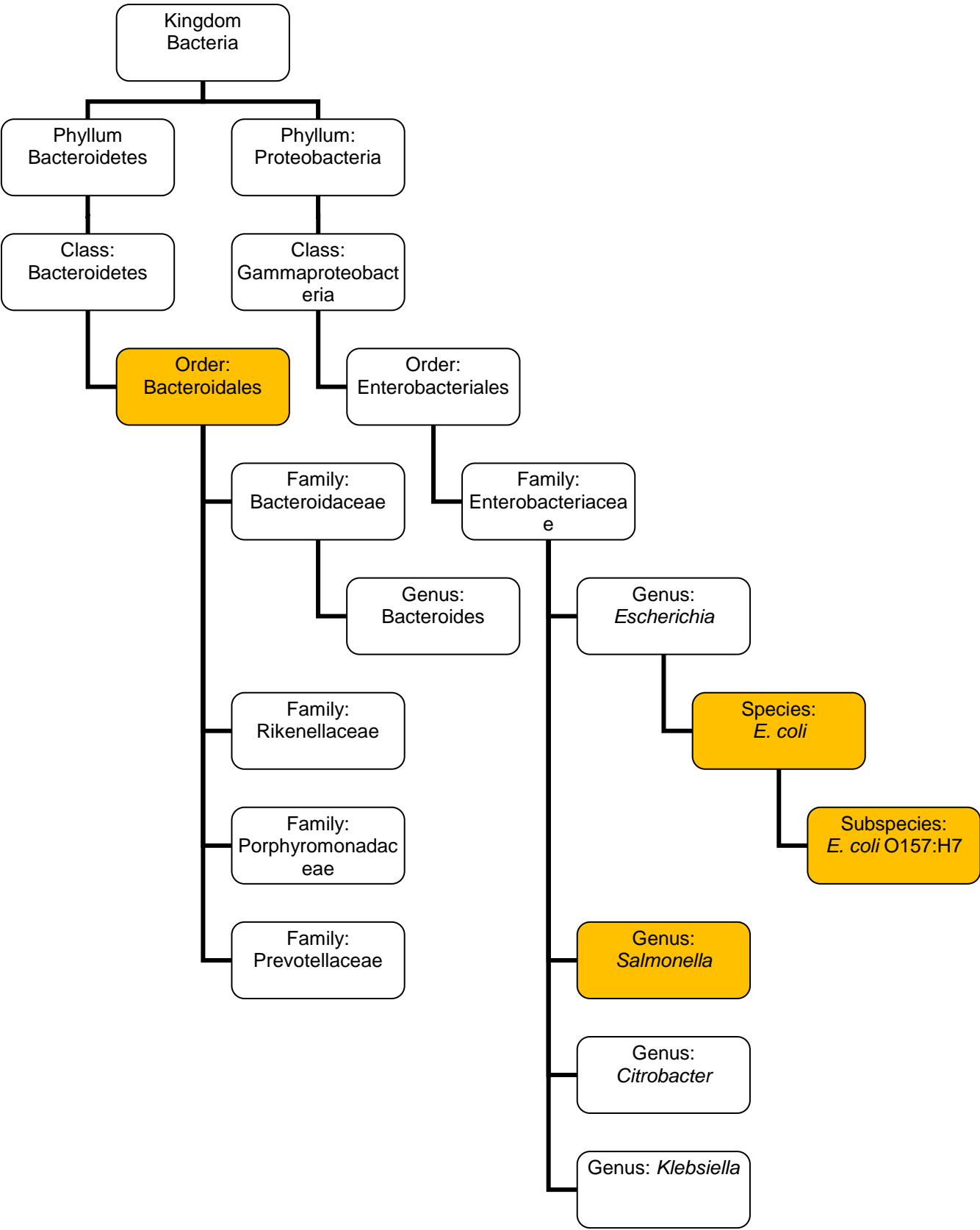


Figure 11. Family tree: Domain Eukaryota

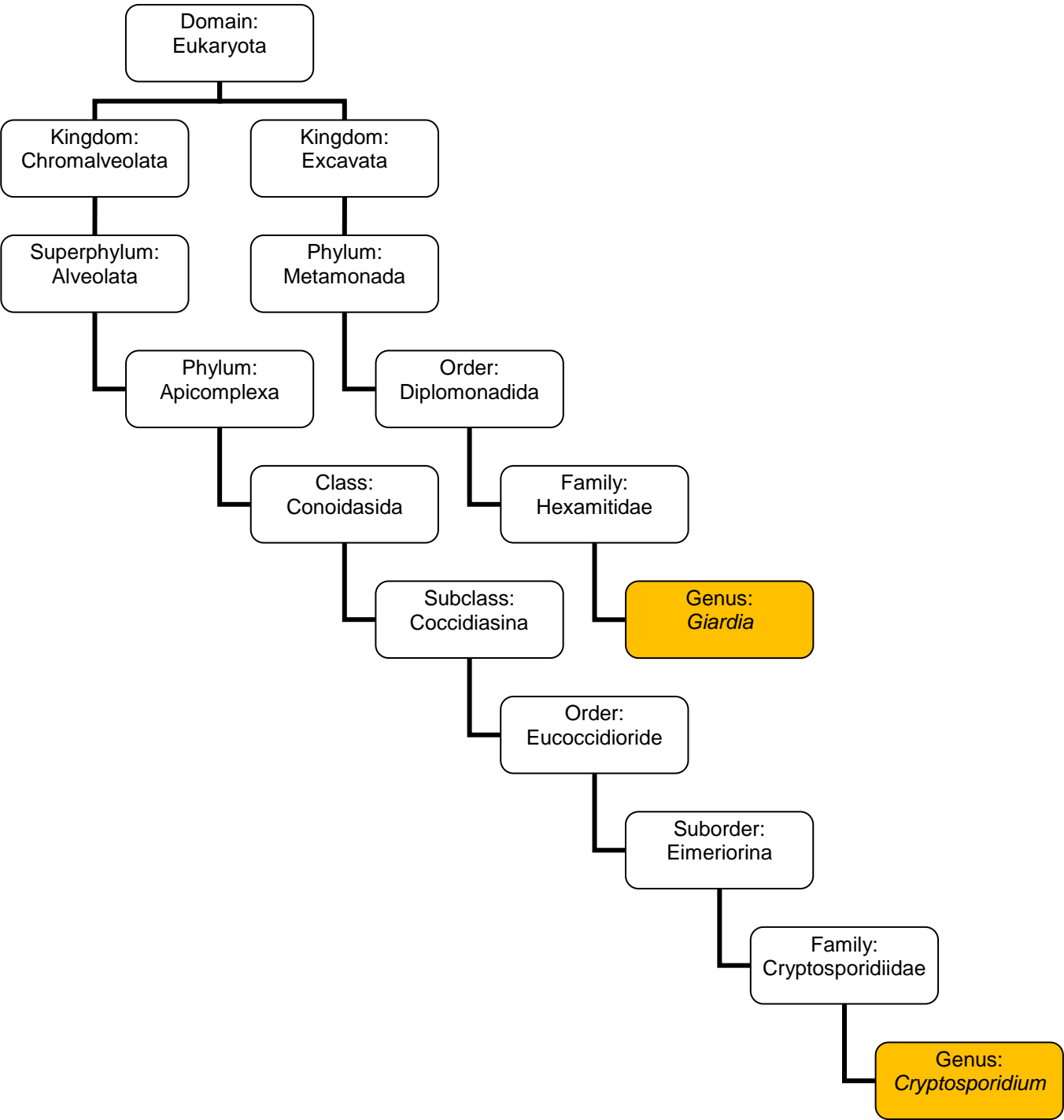


Table 17. Glossary

Term	Definition
ABI Prism 7000	Manufactured by Applied Biosystems, the ABI PRISM 7000 Sequence Detection System can be used to determine the absolute quantity of a target nucleic acid sequence in a test sample by analyzing the cycle-to-cycle change in fluorescence signal as a result of amplification during PCR.
Agglomerates	Clusters of microorganisms as a result of a change in the physical or chemical properties of microbial cells
Assay	A method to analyze or quantify a substance in a sample
Primer	A primer is a strand of nucleic acid that serves as a starting point for DNA synthesis
Basin Plan	Water Quality Control Plan for the Sacramento River and San Joaquin River Basins
BPW	Buffered Peptone Water: Pre-enrichment medium which allows for repair of cell damage and facilitates the recovery of <i>Salmonella</i> .
BSA	Bovine Serum Albumin
Calfed	A group of state and federal agencies that is working to develop long-term solutions to the problems affecting the San Francisco Bay/Sacramento-San Joaquin River Delta (Bay-Delta system). The solution-finding effort focuses on ecosystem quality, water supply reliability, water quality, and vulnerability of Delta levees channels to natural disasters
Citrobacter	Ubiquitous opportunistic pathogen. Rarely the source of illnesses, except for infections of the urinary tract and infant meningitis and sepsis. Can be detected in fecal coliform tests.
Community Development	Areas of potential residential influences to water quality
Confined Animal Feeding Operation	Agricultural operations where animals are kept and raised in confined situations. AFOs congregate animals, feed, manure and urine, dead animals, and production operations on a small land area. Feed is brought to the animals rather than the animals grazing or otherwise seeking feed in pastures, fields, or on rangeland. Additional definition can be found at http://www.epa.gov/region07/water/cafo/ .
<i>Cryptosporidium</i>	A protozoan of the genus <i>Cryptosporidium</i> that is an intestinal parasite in humans and other vertebrates and sometimes causes diarrhea that is especially severe in immunocompromised individuals. <i>Cryptosporidium</i> is one of the major waterborne pathogens.
CT-SMAC II	Macconkey Sorbitol agar, which is a selective medium used in Polymerase Chain Reaction for the isolation and differentiation of <i>E. coli</i> O157:H7
DO	Dissolved Oxygen: Concentration of oxygen dissolved in the water body. Important measure for determining the ability for the water body to support aquatic life.
<i>E. coli</i>	<i>Escherichia coli</i> : A bacillus found in the gastrointestinal tract of warm blooded animals, including humans and existing as numerous strains, some of which are responsible for diarrheal diseases.

Term	Definition
Stx 1/Stx 2	The two major groups of shiga toxin families The most common sources for Shiga toxin are the bacteria <i>S. dysenteriae</i> and the <i>Shigatoxigenic group of Escherichia coli</i> (STEC), which includes serotype O157:H7 and other enterohemorrhagic <i>E. coli</i> (EHEC).
EBMUD	East Bay Municipal Utility District: Water supply District supplying water to customers in the East San Francisco Bay area, receiving water from the Mokelumne and American Rivers.
Enrichment	Bacteria enrichment are procedures to enrich targeted bacterium in samples thus increase the probability of detection of the bacterium
FDA	Food and Drug Administration
Fecal Coliform	A subset of total coliform bacteria that are generally more fecal-specific in origin. Generally not harmful, but may indicate the possible presence of pathogenic bacteria.
Filtration: Cross-flow	Feed water is recycled and water flow is parallel to the membrane. Only a small part of the feed water is used for permeate production.
Filtration: Dead-end	All the water that enters the membrane surface is pressed through the membrane
gDNA	Genomic Deoxyribonucleic Acid: Chromosomal DNA that is the biological information of heredity which is passed from one generation of organism to the next.
IDEXX Colilert® QuantiTray System	Quantitative, statistical water quality test for concentration of total coliform and <i>E. coli</i> . Manufactured by IDEXX.
ILRP	Irrigated Lands Regulatory Program: Program initiated by the California State Water Resources Control Board and its Regional Boards to regulate discharges from irrigated agricultural lands. Its purpose is to prevent agricultural discharges from impairing the waters that receive the discharges.
IMS	Immunomagnetic separation: Enrichment method selective for <i>E. coli</i> O157 cells against non-O157, using an external magnetic source to capture magnetic particles.
Inhibitor	Substances that restrains, blocks, or suppresses PCR reactions.
Integrator Site	Sites located near discharge points of large watershed characterized by heterogeneous land uses. Most Integrator Sites are on major streams with drainage basins that include a substantial portion of the Study Unit area.
Irrigated Agriculture	A significant source of surface water and groundwater nonpoint source pollution. Pollution problems include sediment, nutrients, pesticides, salinity, trace elements, pathogens, and temperature.
Klebsiella	Bacteria common in both the environment and human sources. Can be detected in fecal coliform tests.
Land Use Riparian Vegetation	Marsh lands, tules and sedges, Natural high water table meadow, Trees, shrubs or other larger stream side or watercourse vegetation, Seasonal duck marsh, dry or only partially wet during summer, Permanent duck marsh, flooded during summer
Land Use: Barren and Wasteland	Dry stream channels, Mine tailing, Barren land, Salt flats, and dunes.
Land Use: Commercial	Offices, retailers, etc.; Hotels; Motels; Recreation vehicle parking, camp sites; Institutions (hospitals, prisons, reformatories, asylums,

Term	Definition
	etc., having a reasonably constant 24-hour resident population); Schools (yards to be mapped separately if large enough); Municipal auditoriums, theaters, churches, buildings, and stands associated with race tracks, football stadiums, baseball parks, rodeo arenas, amusement parks, etc.; Miscellaneous high water use
Land Use: Deciduous Fruits and Nuts	Apples, Apricots, Cherries, Peaches and Nectarines, Pears, Plums, Prunes, Figs, Miscellaneous deciduous, Almonds, Walnuts, Pistachios
Land Use: Field Crops	Cotton, Safflower, Flax, Hops, Sugar beets, Corn (field and sweet), Grain sorghum, Sudan, Castor beans, Beans (dry), Miscellaneous field, Sunflowers.
Land Use: Grains and Hay Crops	Barley, Wheat, Oats,, Miscellaneous and mixed grain and hay
Land Use: Idle	Land not cropped the current or previous crop season, but cropped within the past three years, New lands being prepared for crop production.
Land Use: Industrial	Manufacturing, assembling, and general processing; Extractive industries (oil fields, rock quarries, gravel pits, rock and gravel processing plants, etc.); Storage and distribution (warehouses, substations, railroad marshalling yards, tank farms, etc.); Saw mills; Oil refineries; Paper mills; Meat packing plants; Steel and aluminum mills; Fruit and vegetable canneries and general food processing; Miscellaneous high water use (to be used to indicate a high water use condition not covered by other categories.); Sewage treatment plant including ponds; Waste accumulation sites (public dumps, sewage sludge sites, landfill and hazardous waste sites, etc.); Wind farms, solar collector farms, etc.
Land Use: Native Vegetation	Grass land, Light brush, Medium brush, Heavy brush, Brush and timber, Forest, Oak grass land
Land Use: Pasture	Alfalfa and alfalfa mixtures; Clover; Mixed pasture; Native pasture; Induced high water table native pasture; Miscellaneous grasses (normally grown for seed); Turf farms.
Land Use: Residential	Single and multiple family units, including trailer courts; Single family dwellings with lot sizes greater than 1 acre up to 5 acres (ranchettes, etc.); Single family dwellings with a density of 1 unit/acre up to 8+ units/acre; Multiple family (apartments, condos, townhouses, barracks, bungalows, duplexes, etc.); Trailer courts.
Land Use: Rice	Rice
Land Use: Semi-agricultural & Incidental to Agriculture	Farmsteads, Livestock feed lots, Dairies, Poultry farms
Land Use: Truck, Nursery and Berry Crops	Artichokes; Asparagus; Beans (green); Cole crops; Carrots; Celery; Lettuce; Melons, squash, and cucumbers; Onions and garlic; Peas; Potatoes; Sweet potatoes; Spinach; Tomatoes; Flowers, nursery and Christmas tree farms; Mixed (four or more); Miscellaneous truck; Bush berries; Strawberries; Peppers (chili, bell, etc.); Broccoli; Cabbage; Cauliflower; Brussels sprouts
Land Use: Urban	Residential, commercial, and industrial

Term	Definition
Land Use: Urban Landscape	Lawn area - irrigated, Golf course - irrigated, Ornamental landscape (excluding lawns) - irrigated, Cemeteries - irrigated, Cemeteries - not irrigated
Land Use: Vacant	Unpaved areas (vacant lots, graveled surfaces, play yards, developable open lands within urban areas, etc.); railroad right of way; Paved areas (parking lots, paved roads, oiled surfaces, flood control channels, tennis court areas, auto sales lots, etc.); Airport runways.
Land Use: Vineyards	Table grapes; Wine Grapes; Raisin grapes
Land Use: Water Surface	Lakes, reservoirs, rivers, canals, etc.
MP	Monitoring Plan: Defines what to monitor and how the monitoring will be done. It includes information needs, indicators, and methods, spatial scale and locations, timeframe, and roles and responsibilities for collecting data.
MPN	Most Probable Number: Method of getting quantitative data on concentrations of discrete items from positive/negative (incidence data)
MQO	Method Quality Objective: Acceptance criteria for the quality attributes such as precision, accuracy, and sensitivity.
MST	Microbial Source Tracking: Approach or approaches intended to identify the fecal sources impacting a water system.
NWS	National Weather Service
Pathogen	An agent capable of causing disease.
PCR	Polymerase chain reaction: Scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
PMA	Propidium Monoazide, is a membrane-impermeant dye that selectively penetrates cells with compromised membranes, which can be considered dead. Once inside the cells, PMA intercalates into the DNA and can be covalently cross-linked to it, which strongly inhibits PCR amplification.
Precipitation, accumulated	A sensor type where the field measuring device accumulates precipitation during the water year. Some stations accumulation tanks periodically dump the accumulated precipitation to make room for more precipitation. This may cause the value transmitted to jump backward several inches. The value usually accumulates or gets larger until it is reset. A reset may occur if a technician visits the site or it is near the beginning of the season. The dates that designate a season varies according to different agencies (i.e. July-June, October-September). Generally, this sensor type is used for real-time collection duration of hourly or event data.
Precipitation, incremental	A sensor type where the value is either calculated from real-time data or manually entered from an observers report. Generally, this sensor type is used for daily and monthly data.
Presumptive positive	Preliminarily determined to be positive but needs to be confirmed with further tests.

Term	Definition
QAPP	Quality Assurance Project Plan: Documents the planning, implementation, and assessment procedures for a particular project, as well as any specific quality assurance and quality control activities. It integrates all the technical and quality aspects of the project in order to provide a "blueprint" for obtaining the type and quality of environmental data and information needed for a specific decision or use.
qPCR	Quantitative real time PCR: A procedure in which the PCR reaction is tracked as it progresses, by monitoring the accumulating signal that is provided by a fluorescent dye released during each PCR cycle
rfbE	rfbE is a gene responsible for biosynthesis of the O157 antigen of <i>E. coli</i>
RV	Rappaport Vassiliadis broth, is used for the enrichment and selective isolation of <i>Salmonella</i> spp.
SC	Specific Conductance
SLOD	Sample Limit of Detection
SWAMP	Surface Water Ambient Monitoring Program
TaqMan PCR Mastermix	TaqMan PCR Mastermix is a commercial available (Applied Biosystems) solution to minimize set-up times of PCR.
Tryptic Soy Broth	A general purpose medium used for the cultivation of a wide variety of bacteria
USACE	US Army Corps of Engineers
USBR	US Bureau of Reclamation
USEPA	United States Environmental Protection Agency
Water Quality Criteria: <i>E. coli</i> (Basin Plan)	400 MPN/100ml, single sample
Water Quality Criteria: <i>E. coli</i> (EPA)	USEPA Bacterial Water Quality Standards for Recreational Waters Designated Beach: 235 MPN/100ml Moderate Use: 298 MPN/100ml Light Use: 409 MPN/100ml Infrequent Use: 575 MPN/100ml
XLD	Xylose Lysine Deoxycholate, is a selective growth medium used in the isolation of <i>Salmonella</i> .. It has a pH of approximately 7.4, leaving it with a bright pink or red appearance due to the indicator phenol red